THE UNIVERSITY OF ALBERTA

SOME MORPHOLOGICAL AND PHYSIOLOGICAL EFFECTS OF (2-CHLOROETHYL)TRIMETHYLAMMONIUM CHLORIDE (CCC) ON CERTAIN SPECIES OF THE GRAMINEAE

A DISSERTATION

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES

IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE

OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF PLANT SCIENCE

BY

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EDMONTON, ALBERTA

OCTOBER, 1964

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, the thesis entitled "Some morphological and physiological effects of (2-chloroethyl)trimethylammonium chloride (CCC) on certain species of the Gramineae" submitted by Robert F. Norris in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

ABSTRACT

This thesis reports effects of (2-chloroethyl)trimethylammonium chloride (CCC) on the yield of field-grown grasses, on the morphological development of some selected grass species and on the chlorophyll and native auxin content of grass seedlings.

In field trials involving prairie grasses and a stand of cultivated brome grass (*Bromus inermis* Leyss.), spraying with CCC under 'normal' Alberta summer conditions did not have any significant effect on total dry matter yield. It appeared possible that during drought conditions the use of CCC might permit continued plant growth, resulting in increases in yield. Greenhouse experiments also indicated that treatment of plants with CCC might increase their ability to recover from clipping, which might sometimes be of agricultural value.

In greenhouse experiments to determine the effects of CCC on the morphological development of Kentucky bluegrass, (*Poa pratensis* L.), creeping red fescue (*Festuca rubra* L.), brome grass and the three cereal species, wheat (*Triticum vulgare* Vill), oats (*Avena sativa* L.) and barley (*Hordeum vulgare* L.), height of CCC-treated plants was less than that of the controls. Generally those species normally producing long stems and considerable length of rhizome were affected more than those normally having shorter stems and a bunch type growth. The length of rhizome produced was reduced as a result of treatment of the plants with CCC, the magnitude of this effect usually being slightly greater than that on stem elongation. The production of tillers was generally increased at low doses of

CCC, but frequently the high doses, such as 20 and 40 lbs./a., caused considerable retardation of growth which either prevented an increase or caused a reduction in tillering. Leaf number per apical meristem was not significantly affected, consequently the total leaf number per plant followed a pattern similar to that of the effect of CCC on tillering.

Total dry matter yield was usually slightly increased by low doses of CCC, and decreased at the higher ones, the magnitude of the effect varying from species to species. Root: shoot ratios, calculated on the basis of dry weight, tended to show a slight increase after treatment of the plants with CCC, but difficulties in obtaining accurate measurements of root dry weight somewhat limited the value of these data, and results obtained from an experiment to study root development using special 'bottomless' pots proved to be only slightly more convincing.

There appeared to be a greater response to CCC-treatment by plants grown during the winter than those grown during the summer. An experiment involving Kentucky bluegrass grown in a growth chamber showed no significant interaction between four light intensities and CCC treatment. However, some of the results obtained for the production of chlorophyll by CCC-treated corn seedlings indicated that day length may be more critical than light intensity in determining the magnitude of the effect of CCC.

In physiological studies with corn (_Zea mays_ L.) and wheat seedlings, corn had a decreased water content when grown in high concentrations of CCC, and dark-grown wheat appeared

to have increased dry matter content and hence probably a reduced respiration rate when grown in solutions of this chemical.

CCC-treated plants were generally of a darker green than untreated ones. Chlorophyll content of brome leaves from treated plants was significantly greater than that of leaves from untreated plants. Corn seedlings grown in CCC solutions produced more chlorophyll than those grown in water, and this increase appeared to be due to increased protochlorophyll production.

Wheat seedlings grown in CCC solutions contained less free tryptophan and less free indoleacetic acid (IAA) than those grown in water. The amount of an unidentified indole compound (probably IAA or tryptophan) in oats, whose growth was much less responsive to the chemical treatments than was that of wheat, was not affected to any great extent by CCC. This finding may have some bearing, as well, on an explanation of the differences encountered among the responses of the other species included in this research.

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INTRODUCTION

The continuing search for knowledge of the action of applied chemicals in the control of plant growth is of fundamental, and practical agricultural, importance. This aspect of plant physiology and biochemistry has application to production and quality of plants which may be affected directly by the use of appropriate growth regulators; some examples are in the promotion of the rooting of cuttings, control of pre-harvest drop of tree fruits, production of parthenocarpic fruits and for the breaking of seed dormancy. Crops can also benefit indirectly as a result of the development and proper use of herbicides for selective control of associated weeds.

Elucidation of the mechanisms involved in growth regulation has been one of the objectives of the plant physiologist. Went, in the 1920s, discovered that plants contained substances that exhibited hormone-like properties, the auxins. Since then the field of plant growth regulation has grown to encompass not only the naturally occurring growth regulators such as indoleacetic acid, but also the many synthetic compounds that have been found to effect plant growth regulation. The development of these synthetic growth regulators has taken place in the last three decades and has placed a powerful tool at the disposal of the agricultural research worker.

This treatise reports the research dealing with some of the effects of one such chemical, (2-chloroethyl)trimethylammonium chloride (CCC), as a crop plant growth regulator on certain members of the Gramineae. At the outset of this investigation, discovery of the growth regulating properties of

CCC was relatively recent. Consequently knowledge concerning its effects on plants was limited, and a broad based exploratory approach was adopted for the initial phases of the work.

Three major aspects involving CCC were investigated. 1). Its effects on some forage grasses grown under field conditions in Alberta. 2). Its effects on the morphological development of certain individual grass species. 3). Its effects on chlorophyll production and the level of native auxins in grass seedlings.

LITERATURE REVIEW.

The earliest record of plant growth regulation by compounds related to (2-chloroethyl)trimethylammonium chloride (henceforth designated by the abbreviation CCC) was by Schreiner and Reed (45) in 1908, who found that 25 ppm. of (ethylene)trimethylammonium bromide injured wheat seedlings, and that 250 ppm. was toxic. No further work on this group of chemicals as plant growth regulators was reported until 1960. At this time Tolbert (52) published the results of a wheat stem bioassay using CCC and many compounds that are structurally related to it. CCC proved to be one of the most active.

CCC, which has also been known as chlorocholine chloride (hence CCC) and is now marketed commercially under the name 'Cycocel'*, is produced by the reaction between trimethylamine and 1,2-dichloroethane; it is a crystalline solid and is readily soluble in water. It is not phytotoxic unless applied at extreme dosages; and it has been found to have a relatively low mammalian toxicity as well (50). The empirical formula for CCC is $C_5H_{13}Cl_2N$ (52), and the structural formula can be represented as:

$$\left[\begin{array}{ccccccc} & & H & & H & & CH_3 \\ & & | & & | & & | \\ Cl & - & C & - & C & - & N - CH_3 \\ & & | & & | & & | \\ & & H & & H & & CH_3 \end{array}\right]^{+} .Cl^{-}$$

At the present time the occurence of native CCC in plants has not been established. In 1961 substances were isolated from tomato plant extracts that could not then be distinguished

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* - Cyanamid of Canada Ltd., Rexdale, Ontario.

from CCC (36); more recent work has shown that these were not CCC but the related compound choline and some of its derivatives (35,42). Other trimethylammonium compounds are known to occur in plants infected with species of the fungus Tilletia (the bunts)(40), but none of these appears to possess growth regulating properties; neither does betaine, a closely related compound that also occurs in plants (40,52).

In most of the published work CCC was supplied to plants as a soil drench or by direct incorporation into the soil. In some cases nutrient solutions with CCC added have been used (7,16,22,31,43), and seed treatment has been successful on wheat (37,38). A few workers have reported using foliar sprays; results equal to those using the other techniques were obtained for poinsettias (6), but reduced effects were obtained for tomatoes and peppers (51) and for chrysanthemums (31).

CCC alters the morphological development of treated plants. The most obvious effect is a decrease in internode length, resulting in shorter, and usually more compact, plants. This was first observed by Tolbert (52) when measuring the stem length of wheat plants between the base of the first and the base of the second leaf blade in the previously mentioned bioassay. Reduction in internode length has now been observed in many plants; some of these are wheat (53), tomatoes (51,59), poinsettias (6,31), mustard, radish and tobacco (19), azaleas (48), chrysanthemums (3,31) and other plants (4,50). A thickening of the stem usually occurs, its magnitude depending on the degree of stem reduction (51,53,59). As a result of this effect on stem length, CCC has become known as a growth retardant. In recent years several other groups of chemicals have

been found to exhibit similar plant growth regulating properties, but these are either effective only on a relatively restricted range of plants, or they are toxic when used at higher concentrations or when applied as foliar sprays. One other compound that is structurally related to CCC, allyl trimethylammonium bromide (AMAB), has also been used by some workers. Its effects are similar to those of CCC and the inference has been drawn from some of the experiments conducted using AMAB that CCC, although not tested, would have had similar effects (31).

It was immediately obvious that the decrease in internode elongation was an effect opposite to that produced by gibberellins. Much recent work has indicated that the action of CCC is related to that of the gibberellins (16,18,22,32,53,60). Some workers suggest that it should be known as an 'antigibberellin' (22,32). This will be discussed later.

Depending on the type of plant, a second effect of CCC on morphological development, is an increase in branching, or, with grasses, tillering. Wheat treated with CCC showed an approximately three to four fold increase in tillers per plant (53) and tomato plants had a more rapid and sturdy development of lateral shoots (59).

CCC affects the colour, shape, size and production of leaves, the magnitude of these effects varying considerably between species. Wittwer and Tolbert (59) reported that the leaves of treated tomato plants were a darker green, more deeply lobed, thicker and larger than those of untreated plants. Working with chrysanthemums, Lindstrom and Tolbert (31) obtained similar results. Greenhouse work by Humphries (19) with

mustard plants, showed that CCC-treatment increased the total leaf area per plant, either by increased leaf production on lateral branches or by increased size of main stem leaves. It should be noted that this effect on mustard is similar to that produced by exposure of the plants to short days (mustard is a short-day plant). The possiblity that the effect of CCC is mediated by light intensity or duration will be considered later. For tobacco Humphries also reported that leaf length was reduced by treatment of the plants with CCC, but that leaf dry weight was not altered, indicating that the leaves must have been thicker than those of untreated plants. Subsequent work by Halevy and Monselise (18) confirmed that CCC-treatment produced thicker leaves. Punching discs from bean leaves showed that those from the leaves of CCC-treated plants had a consistently greater dry weight than those from the controls. They also found that CCC altered the pattern of translocation from the leaves during the night. Untreated plants had a maximum leaf dry weight at midnight, decreasing after that time, whereas leaves from CCC-treated plants attained maximum dry weight about one hour after sunset, followed by a steady decline. At no time, however, did the dry weight of leaves from CCC-treated plants drop as low as that of leaves from the controls. This effect on translocation pattern could be partially reversed by the application of gibberellin, and this observation furnishes another instance of interaction between CCC and gibberellin. The only monocotyledonous plant to be studied intensively regarding the effects of CCC has been wheat. Tolbert (53) reported that the leaf number was not affected by this chemical, but that the leaves from treated

plants were a darker green, shorter and wider than those of untreated plants. He also noted that treated plants remained greener longer at the time of ear ripening.

This intensification of green colour in leaves has been found to be one of the first visible effects to develop after treatment of plants with CCC, and has been observed for several species. When foliar sprays were used, chlorotic areas developed on the leaves receiving the spray (31), but these areas recovered. Insofar as can be ascertained the only available data concerning the effect of CCC on chlorophyll content are those of Humphries for tobacco (19). Leaves from treated plants contained approximately seven percent more chlorophyll on a per leaf basis and approximately 65 percent more on a per unit dry weight basis than those of the controls, when 10^{-3} M CCC was used in the culture solution. He also reported that CCC effected a redistribution of nitrogen, there being relativley more in the leaves than in the stems of treated plants. It is possible that the changes in chlorophyll content can be related to this. These results again demonstrated that CCC causes effects opposite to those of gibberellin, which has been found to decrease the amount of chlorophyll in leaves (39,61). Chlorophyll is not the only pigment affected; anthocyanin production was increased in Bryophyllum daigremontianum after treatment of the plants with CCC (64).

A fourth aspect of CCC on plant morphology concerns its effects on the development of roots. Wittwer and Tolbert (59) reported that the dry matter yield of tomatoes was increased by CCC-treatment, more so for the roots than the tops, resulting in an increased root to shoot ratio. A similar effect had

previously been observed, by Fletcher and Renney (8), for tomatoes, beans and bent grass. This view was not supported by Lockhart (32) who, in trying to locate the site of action of several growth retardants, came to the tentative conclusion that, at least in 'Pinto' beans, CCC exerted most of its effect on the stem and little on the roots. The possibility exists that the observed increases in root to shoot ratio were due to a reduction in top growth without any alteration of root growth. At the time of writing no other data appear to have been published concerning dry matter production of roots after treatment of plants with CCC. Cathey and Stuart (5) investigated the effects of three growth retardants on the rooting of chrysanthemum cuttings; CCC was found to be relatively inactive in this respect. Libbert and Urban (30), however, studying the rooting of cuttings of Convolvulus sepium, found that CCC-treatment increased the number of roots produced. They explained this result on the assumption that CCC is an antigibberellin, and that naturally occuring gibberellin-like substances that have been found to occur in Convolvulus normally suppress adventitious root production. They claimed that CCC-treatment had reduced the level of these substances to a point at which they were no longer inhibitory. This again suggests that the actions of CCC and gibberellins are related.

In some of the species that have been investigated CCC appeares to modify the flowering response, although the nature of the effect varies widely between species, and appears to be related to both the photoperiodic response and to gibberellin. In regard to petunias and chrysanthemums, Cathey and Stuart (5) reported that CCC-treated plants did not initiate flowers after

exposure to long days (they normally flower under short days); 'Biloxi' beans also remained vegetative under similar conditions (60). Thus, in these species, CCC did not alter the flowering pattern. A delay in the flowering of chrysanthemums under conditions normally favorable for flowering has, however, been reported by Lindstrom and Tolbert (60). A side effect of potential use to commercial florists was that flowers from CCC-treated plants were found to keep better than those from untreated plants. Zeevaart and Lang (64) showed that CCC applied at one gramme per plant completely suppressed the flowering of Bryophyllum daigremontianum when grown under conditions otherwise favorable for flowering (long day to short day shift). Gibberellin A_3 overcame this suppression of flowering, although ten times as much was required to overcome the effect of internode shortening that had also occurred. Recently, Zeevaart (63) demonstrated a similar response in Pharbitis nil. He tentatively concluded that this suppression was due to decreased rate of cell division, which did not allow flower bud development to take place even after the plumule had received the floral stimulus. Wittwer and Tolbert (59), working with tomatoes, reported that flowers were set earlier and at lower nodes after treatment of the plants with CCC; this was confirmed by Tiessen (51), both for tomatoes and for peppers. Probably the greatest effect of CCC on flowering has been on azaleas; Stuart (48) reported that following CCC-treatment vegetative shoots rapidly initiated flower buds under conditions normally preventing it. Tolbert (53) reported the flowering of wheat to be affected only to the extent that CCC caused a delay of about four days. A completely different

response has been observed for cucumbers; Wittwer and Bukovac (58) found that CCC-treated plants had more female flowers than the controls. This effect is also opposite to that of gibberellin, and it is of potential importance commercially.

The dry matter yield of plants after treatment with CCC is usually not affected or is slightly reduced, depending on the concentration used and the type of plant. When large quantities of CCC are applied, the resulting severe retardation leads to a reduced accumulation of dry matter. There have been several reports on dry matter yield; Tolbert (53) found no significant effect on wheat, Lindstrom and Tolbert (31) reported little effect of CCC on the dry matter yield of poinsettias and chrysanthemums, but Wittwer and Tolbert (59) found an increase for tomatoes. This latter result was not confirmed by Tiessen (51), who reported that CCC had no effect or caused a reduction in the dry weight of tomatoes and peppers; it is possible that observed differences in yield of plants after treatment with CCC may be due the time of year at which the experiments were carried out. The only critical study of the effects of CCC on plant growth rate and dry matter yields has been the work on mustard and radishes by Humphries (19). He reported a decrease in the dry matter yield of both species after treatment of the plants with CCC, this being related to a lowering of the net assimilation rate. He suggested that this might have been caused by an increased mutual shading of the leaves, either by increased leaf area or by decreased internode length; or by what he termed a reduced photosynthetic 'sink', due to lowered stem size. With regard to fruit yield, Tiessen (51) found a trend towards decreased yield in peppers

and tomatoes, except for an increase for peppers when grown at high night temperatures following foliar application of CCC. This result was similar to that obtained for untreated plants grown under cool night conditions. Little effect on grain yield of wheat was recorded for CCC (53); possibly yield was raised slightly through increased kernel size concurrent with no change in number per ear.

References have already been made to the suggestion that the action of CCC is related to light and temperature. CCC also appears to exert considerable modification on the response of plants to other extreme environmental factors, such as soil pH and salt concentration. Tolbert (53) noticed that the effect of CCC was less pronounced in the summer than in the winter and he stated that CCC-treated plants characteristically appear like untreated plants grown under high light intensity. A similar effect was also reported for fern prothalli by Kelley and Postlethwait (21), who stated that CCC-treatment of the prothallus closely resembled that brought about by exposure of untreated prothalli to blue light. It is interesting to note that Tolbert (53) found that CCC was without effect on wheat grown in darkness or at light intensities of less than 10 ft-c.. In addition to the possible interaction with light intensity, CCC-treatment made a few sensitive plants grown in long photoperiods look as if they had been grown in short ones (19,53,60). Thus, at the time of writing, the nature of the interaction between the effects of CCC and light is still uncertain, some plants appearing to respond to differences in light intensity and others to day length (possibly combined with light intensity), these dif-

ferences in response applying to both vegetative growth and flower initiation. It would appear that CCC also affected the response to temperature; treated plants grown under warm night conditions looked like untreated ones from cooler temperatures (51).

Lindstrom and Tolbert (31) mentioned that CCC-treated chrysanthemum plants did not wilt as quickly as untreated ones, and Cathey and Stuart (5) stated that CCC-treated petunias suffered much less under conditions of water stress than did untreated ones. Using 'Brittle Wax' bean plants, Halevy and Kessler (17) were able to demonstrate that wilting did not occur in treated plants until 30 days after watering ceased, whereas the controls wilted in five days. They also found that there was less water left, at the wilting point, in the soil in which treated plants had been growing than in that in which the control plants had been growing, indicating that CCC had lowered the effective wilting point. As would be expected from the above results, CCC-treated plants had a significantly higher dry weight under these conditions.

Work by Miyamoto (37) has shown that CCC-treatment greatly increased the tolerance of wheat seedlings to high salt concentrations. By adding ammonium nitrate to the soil in the pots and measuring the percentage wilting of the wheat seedlings in it, he demonstrated an increase in tolerance of approximately 60 percent over that of the controls, at all the levels of ammonium nitrate used. He also found an increased tolerance to Knopp's solution when used above the recommended level. A similar increase in tolerance to high salt concentrations after treatment of plants with CCC

had previously been demonstrated for soybean plants by Marth and Frank (34), using excessive rates of commercial 5-10-5 fertilizer. Also, Miyamoto (38) subsequently reported that CCC-treatment increased the tolerance of wheat seedlings to extremes of pH. At pHs ranging from 11.18 to 11.98 (achieved by the addition of NaOH to the soil) he recorded 5 to 30 percent increases in tolerance for CCC-treated seedlings, and at pHs from 4.35 to 3.24 (achieved by the addition of H_2SO_4 to the soil) he obtained from 10 to 40 percent increases in comparison with untreated plants exposed to the respective pHs. He did not attempt to explain any of these results, but it would appear possible that one basic change in the physiology of the plants could account for both the above described effects of CCC, and also that of drought resistance. Any method by which these effects could be related to those observed on morphology would, however, remain a matter for speculation.

Recently, Van Emden (55) demonstrated that CCC-treated brussel sprouts were not as heavily infested with cabbage aphids as the control plants. In what would appear to be a completely different field, Sinha and Wood (46) reported that CCC increased the resistance of susceptible tomato varieties to 'wilt' disease caused by the fungus Verticillium; in this case it was shown that the resistance was due to changes in the host, as CCC-treated cultures of the fungus grew better than untreated ones. In both these reports the authors suggested that CCC might have altered the host plant physiology, in such a way that the parasite could no longer compete as successfully. Related to these findings, Rawlins (see review by Cathey (4))

demonstrated that treatment of tobacco discs with CCC lowered the rate of multiplication of mosaic virus. It is possible that the metabolic changes suggested above could be related to those that must have occurred to increase the tolerance of the wheat seedlings to the extreme environmental factors that have been described previously.

The effects of CCC on non-vascular plants have received little attention. The work by Kelley and Postlethwait (21) using fern prothalli has been referred to already. They reported that CCC increased the number of cells, total area and number of rhizoids per prothallus; it also affected the response to light as described earlier. Conrad and Saltman (7) studied the effects of the closely related AMAB on growth of the alga Ulothrix subtilissima. They found that it acted as a growth promotor at low concentrations and as an inhibitor at higher levels; also that its effects could be reversed by gibberellic acid. The only other reference to the effect of CCC on non-vascular plants is that the fungus Verticillium grew slightly better if CCC was added to the culture medium (46), possibly because of the added carbon.

At the time of writing, the results of work reviewed here have led to only one commercial use for CCC; this is for the controlling of poinsettia development. Several of the authors cited previously suggested other possible uses; examples are: controlling of lodging of wheat (53), increase in flowering of azalea (48), substitution for low temperature, enabling the growing of cool-temperature-requiring plants in warmer areas (51) and possibly increasing the resistance of treated plants to pests and diseases (46,55).

Before discussing the mode of action of CCC, its fate in the plant, and in the soil, should be considered. Cathey and Stuart (5) tested it for persistence in the soil, and found it to be rapidly broken down; no activity, as determined by bio-assay, was left after three to four weeks. However, once in-side the plant it appears to be relatively stable. Tolbert (unpublished, see Lindstrom and Tolbert (31)) found that C^{14} labelled (2-bromoethyl)trimethylammonium bromide (BCB), which has effects on plants similar to those of CCC, was metabolized very slowly or not at all. Tolbert, in a personal communicat-ion, wrote that C^{14} labelled CCC was rapidly translocated in plants and that it was not metabolized (C^{14} labelled CCC is not commercially available).

Reduction of internode length immediately poses the quest-ion: does CCC affect cell production, or elongation, or both?. Very little work has been published on this aspect. Sachs and Lang (43) studied shoot histogenesis of *Xanthium* after treat-ment with gibberellin, AMO 1618 (4-hydroxy-5-isopropyl-2-methyl-phenyl trimethylammonium chloride,1-piperidine carboxylate) and maleic hydrazide. AMO 1618 (which is a growth retardant) re-duced subapical cell division to a very low level, but did not affect the apical meristem, which explains how stem shortening can occur without loss of leaf production. Gibberellin was found to be capable of reversing this effect. Several authors have since suggested that this may be the way by which CCC pro-duces its effects. Zeevaart (63) found a reduction of approx-imately 60 percent in cell numbers of the first internode of *Pharbitis nil* seedlings after treatment with CCC at 300 mg./ litre. Recently, Sachs and Wohlers (44) demonstrated that

CCC reduced, or completely inhibited, cell division in callus tissue of carrot, chrysanthemum and geranium; but they found it had no effect on tobacco callus. They did not obtain a reversal of the effect by adding either auxin or gibberellin, and concluded that the action of CCC cannot be explained simply as a reduction in the biosynthesis of these compounds. The result obtained for tobacco callus would appear to cast some doubt on the validity of the tissue culture technique just described when used to supply evidence concerning the action of CCC on intact plants, as Humphries (19) had previously reported development of typical CCC symptoms after treatment of tobacco plants. Results of treating fern prothalli with CCC showed a highly sigificant increase in cell division (21). This again indicates the difficulty in attempting to correlate the results obtained by one method with those obtained by a different one. There have been no reports concerning the effects of CCC on cell size, and if, as will be explained later, the available auxin content of treated plants is reduced, then shorter cells would be expected.

During the presentation of the results of the work with CCC to date frequent mention has been made of the possible interaction, or antagonism, of the action of CCC and gibberellin. In the earliest work, Tolbert (53) noted that application of gibberellin after CCC treatment reversed the effect of the latter. Similarly, it has been shown that CCC applied to gibberellin-treated plants will, at the correct concentration, nullify the gibberellin effect. Examples of this interaction of the effect of CCC and gibberellin have been outlined previously; they included: stem elongation, leaf area and

chlorophyll content, rooting of cuttings and various flowering responses. A further example was supplied by Wittwer and Tolbert (60) in that CCC application greatly suppressed the dormancy-breaking effect of gibberellin on 'Grand Rapids' lettuce seed. Lockhart (32) published evidence to support the theory that CCC acts as an antigibberellin, by partially blocking the system which provides active gibberellins to the growth mechanism. More recent work by Kende, Ninnemann and Lang (22) has shown that CCC suppressed the formation of gibberellic acid by the fungus Fusarium moniliforme when grown under culture conditions. They stated that it is likely that gibberellin synthesis is along similar pathways in higher plants and that the effects of CCC can be explained in terms of inhibition of the biosynthesis of naturally occurring gibberellins; this is exactly opposite to the conclusion drawn by Sachs and Wohlers (44) from their work on callus tissue.

Evidence has also been presented to prove that CCC is not an antigibberellin. Stuart and Cathey (49), in a 1961 review on gibberellins, stated that CCC and related substances should not be called antigibberellins, as there is no relationship in the amounts of these chemicals required to bring about reversal of effects, and also because it does not always compete with gibberellin. This was demonstrated by Kuraishi and Muir (26), who reported that the inhibiting effects of CCC on coleoptile growth were overcome by indoleacetic acid (IAA) treatment, but not by gibberellin; also that CCC-treated 'Alaska' pea stem segments responded to IAA treatment but not to gibberellin. They expanded their work by measuring, using a bioassay, the diffusible auxin in pea stem apices, and reported that a CCC

treatment at 10^{-4}M, by soil drench, reduced the stem length by 10 percent and the diffusible auxin by 50 percent; CCC at 10^{-1}M reduced stem length by 50 percent and the auxin level by 85 percent of that of the controls. From this they concluded that CCC exerts its effect through a lowering of the auxin level in the plant. In the previously mentioned work with CCC on Pharbitis nil, Zeevaart (63) also came to the conclusion that CCC is not a competitive inhibitor of gibberellin, as he found that the same minimal amount of gibberellin A_3 overcame the effects of different levels of CCC on flower inhibition. It has also been pointed out that there is no structural similarity between CCC and gibberellins (49,54,60) and that a straightforward competition would be difficult to support under such circumstances. In a recent review on the physiology of growth retardants, Cathey (4) again emphasised that CCC should not be considered as an antigibberellin, but probably more accurately an antimetabolite.

Work by Halevy (16) has clarified the situation considerably, and offers an explanation for the CCC/gibberellin interaction. Using cucumber seedlings, he measured the peroxidase and IAA-oxidase activity after treating plants with gibberellin or one of several growth retardants, including CCC. Gibberellin was found to decrease the activity of these enzymes, resulting in a higher level of IAA in the plants; CCC (and all other growth retardants, except maleic hydrazide) increased the enzyme activity and thus effectively reduced the level of IAA. Gibberellin and CCC applied simultaneously were antagonistic, one nullifying the effect of the other. From this work Halevy concluded that CCC exerts its effect on plant growth by

interacting with gibberellin on IAA-oxidase (or one of its co-factors and inhibitors) activity, and thus reduces the auxin level in the tissue, resulting in the observed morphological changes. He also is of the opinion that his work supports Lockhart's (32) concept of CCC action, in that it affects a few factors in the growth system, but not directly the bio-sythesis of gibberellin. The concept that auxin is not the only substance involved in plant growth regulation is not new, and Kefford and Goldacre (20) propose that it should be considered as a predisposing agent, interactions with gibberellin and kinetin modifying the plant's response to it. In this way CCC could also be included as a compound which modifies auxin action, through one or several pathways, one of which could be gibberellin. It should be remembered, however, that this is only an explanation of the effects of CCC on stem elongation, and that some of the evidence previously cited indicated possible effects on other, completely unrelated, physiologic processes.

In summarizing the findings concerning effects of CCC reviewed in the preceding pages the following general statements appear to be justified. CCC-treated plants are shorter, a darker green and frequently show greater production of lateral branches in comparison with the untreated plants. The dry matter yields are generally not affected or are slightly reduced by CCC, but the extent of this effect appears to vary considerably from species to species. CCC also appears to modify the flowering behaviour of plants, species differences again affecting the exact nature and the extent of this response. With some species, CCC-treated plants grown under

long, low light intensity, photoperiods appear similar to untreated plants grown in shorter, high light intensity, photoperiods, and for other species the effect is apparently related to light intensity only. A similar response to temperature has also been observed, CCC-treated plants grown in warm temperatures appearing like untreated ones grown in lower temperatures. Some reports indicate that CCC-treated plants show increased tolerance to extremes of environmental conditions such as pH and salt concentration and also to certain pests and diseases. A number of morphological effects of CCC have been found to be opposite to those induced by gibberellin treatment, and it has been suggested that CCC should therefor be known as an 'antigibberellin'. Other workers have reported data showing that CCC is not an antigibberellin, but rather that the two compounds interact in controlling the level of auxin in the plant. The diffusible IAA content of CCC-treated pea stems has been shown to be much lower than that of untreated pea stems, and subsequent work has suggested that this may be due to increased activity of IAA-oxidase, which has been shown to occur after treatment of plants with CCC. Changes in the level of IAA, and possibly related compounds also, might explain some of the observed effects of CCC on plant morphology, such as decreased stem elongation, increased tillering and changes in the response to flowering.

EXPERIMENTAL I. FIELD EXPERIMENTS

Materials and methods

Experiments were conducted at the University of Alberta ranch at Kinsella, Alberta, during the summers of 1961, 1962 and 1963. This ranch is situated in the thin black soil area of Alberta about 100 miles east of Edmonton. It has a relatively low rainfall of approximately 15-17 inches and is a native fescue grassland area, with scattered stands of aspen (*Populus tremuloides* Michx.), and extensive occurrence of wolf willow (*Eleagnus commutata* Bernh.) and snowberry (*Symphoricarpos occidentalis* Hook.). The grassland proper consists of rough fescue (*Festuca scabrella* Torr.), sandberg bluegrass (*Poa secunda* Presl.) and/or Hooker's oat grass (*Helictotrichon hookeri* (Scribn.) Henr.), sedge (*Carex heliophila* Mack.) and to a varying degree two species of sagewort (*Artemisia frigida* Willd. or *A. ludoviciana* Nutt.) as the dominant species. Other species which occur frequently are:

Latin name	Common name	Time of flowering.
Agrostis scabra Willd.	Tickle grass	August
Anemone multifida Poir.	Cut-leaved anemone	May
Anemone patens L. var. *wolfgangiana* (Bess) Koch.	Prairie crocus	April-May
Antennaria nitida Greene	Cats paw	May
Gaillardia aristata Pursh	Gaillardia	August
Geum triflorum Pursh	Prairie smoke	May
Gutierrezia sarothrae (Pursh) Britt. & Rusby	Broomweed	August
Koeleria cristata (L.)Pers.	June grass	June-July
Melampyrum lineare Desr.	Cow wheat	August
Potentilla pennsylvanica L	Pennsylvania cinquefoil	June
Selaginella densa Rydb.	Little club-moss	-
Senecio canus Hook.	Prairie groundsel	June-July
Solidago missouriensis Nutt.	Golden rod	August

Latin name	Common name	Time of flowering.
Stipa comata Trin. & Rupr.	Spear grass	July
Thermopsis rhombifolia (Nutt.) Richards	Golden bean	May-June

Throughout this treatise the following standard abbreviations will be used in conjunction with data concerning treatment of experimental material. References to rates of CCC application (or fertilizer) will be in pounds per acre abbreviated to lbs./a., similarly parts per million to ppm. and total volume applied in Imperial gallons per acre to gal./a.. The generally accepted standard of quoting rates of application as amounts of active ingredient will be adhered to. Data concerning spraying, such as pressure and speed (where applicable) will be abbreviated from pounds per square inch to p.s.i. and miles per hour to m.p.h.. Experiments involving different light intensities will have measurements made in foot candles, abbreviated to ft-c..

Soil analyses were made in 1961 on two Kinsella soil samples (analyses carried out by the Agricultural Soil and Feed Testing Laboratory, University of Alberta, Edmonton). The available nitrogen (N_2) and phosphorus (P) were very low, there being only 21 and 14 lbs./a. present respectively; a good soil is considered to contain not less than 40 lbs./a. of available N_2 and 60 lbs./a. of available P.. Excessive quantities of potassium (K) were found to be present, the available amount being 137 lbs./a. in comparison with a level of 50 lbs./a. considered to be adequate for a good soil. Elephant Brand fertilizer (analysis 16-20-0 of N-P-K) was broadcast at 300 lbs./a. over experimental areas prior to spraying.

Application of the CCC treatment solutions was by a hand-propelled three-wheeled plot-sprayer fitted with a boom having three fan type nozzles giving a five feet wide coverage. At a pressure of 40 p.s.i. (supplied from a compressed air bottle) and at a ground speed of 2.7 m.p.h., 10 gal./a. total volume were delivered for one pass over the plot; this quantity was used as it was considered to be as much as would be feasible if ever used commercially. Spraying was used in preference to soil drenches (or related techniques) as the use of large volumes of liquid is not possible under field conditions.

Two experiments were laid out at Kinsella in the spring of 1961. The first was a randomized block design to test the effect of repeated light CCC applications. A plot size of 20x5 ft., with 3 ft. pathways between blocks, was used. CCC, from a 50 percent (w/v) aqueous stock solution, was made up to provide a rate of 3 lbs./a. when applied from type 8001 E 'Tee Jet' nozzles (Spraying Systems Co., Bellwood, Illinois). Four plots of each of the three replicates were sprayed on June 1st, three of these were resprayed on June 22nd, two on July 12th and one on August 2nd. This resulted in plots receiving a total of 0, 3, 6, 9 and 12 lbs./a. of CCC respectively. Harvesting was by hand clipping two square yards of vegetation from each plot. These samples were stored in polyethylene bags at approximately 3°C.. In order that an estimate could be made of the effects of CCC on different components of the vegetation, each sample was separated into four major groups; 1) grass (including sedges), 2) sageworts, 3) golden rod, and 4) all remaining species. These

separated samples were dried in an oven at approximately 80°C. and weighed.

The second experiment at Kinsella in 1961 was a factorial design intended to test the effect of different dosages of CCC in combination with the effect of spraying date. Three replications were used, laid out as three randomized blocks; plot size was again 20x5 ft.. CCC was applied at 0, 7½, 15 and 30 lbs./a., different plots being sprayed on one of three successive dates. Harvesting and sampling procedure was similar to that for the experiment described previously, except that three square yards were clipped instead of two.

One respraying of part of the second 1961 experiment was the only field work carried out in 1962. Plots from the second spraying date in 1961 were those retreated. A change in the concentration of the stock CCC solution from 50 percent (w/v) to 11.8 percent (w/v) necessitated an increase in the total volume applied per plot from 10 gal./a. to 40 gal./a. in order that the heaviest dose could be applied. This was effected by increasing the pressure to 45 p.s.i. and by travelling over each plot three times at 2.9 m.p.h.. All other procedures were identical to those for the previously described experiments.

In 1963 an experiment was conducted to obtain further information on the effects of several CCC doses applied on three different dates on grassland at Kinsella. A 5x5 latin square design was used and plot size was again 20x5 ft.. The CCC treatments were 0, 5, 10, 20 and 40 lbs./a., with 0.1 percent Tween 20 (manufactured by the Atlas Powder Co. Canada Ltd., Brantford, Ontario) added as a wetter. The

sprayer used for this work was fitted with three 8003 fan nozzles, giving 40 gal./a. at 30 p.s.i. for one passage at 1.25 m.p.h. over each plot. In the early spring, before growth commenced, the whole experimental area was mown and raked clear in an attempt to minimize variation. Harvesting was done by mowing a swath three feet wide and nineteen feet long from the centre of each plot, six inches being left uncut at each end. A Simplicity 7 h.p. garden tractor, with a front mounted cutter bar and collecting basket, was used for the mowing of the composite samples, which consisted mainly of grasses.

In the last field experiment the effects of CCC on a stand of cultivated brome grass (Bromus inermis Leyss.) were investigated at the University of Alberta farm at Ellerslie, Alberta. This farm is situated in the black soil area near Edmonton, which is relatively fertile in comparison with that at Kinsella, and has a somewhat higher rainfall of approximately 18-20 inches. Rows of brome grass, one foot apart and 150 ft. long, were sown into recently tilled land on May 16th 1963. A Simplicity 7 h.p. garden tractor with a seeder attachment was used; this placed the 'seed' approximately two inches below the soil surface at a rate of between 10 and 12 lbs./a.. Hand-weeded plots 20x10 ft. in size were laid out each including 10 rows of brome plants. At the time of spraying, July 10th 1963, the brome plants were about 10 cms. tall, had 5-7 leaves and had commenced producing secondary basal shoots. The rates of CCC and data concerning application were the same as those for the previously described latin square experiment at Kinsella,

except that the larger plots had to be sprayed in two halves. Harvesting consisted of cutting the central three rows from each half of the plot.

Results

Visible effects of CCC on dry prairie grassland were slight. Leaf tip scorch and some chlorosis were observed when high rates were used; no other symptoms distinguished the treated plots from the controls. Blotchy chlorosis of the leaves, accompanied by tip scorch, was observed for the brome grass at Ellerslie within one week of spraying, at rates of 10 lbs./a. or higher. At the time of harvesting this experiment, the plots receiving 20 and 40 lbs./a. of CCC could be visually identified because of reduced height; some leaf tip scorch was also still evident.

Repeated applications of a small amount of CCC did not produce any change in dry matter production of dry prairie grassland at the University of Alberta ranch at Kinsella. Data presented in Table I show that there were considerable

Table I. Effect of repeated light CCC applications on the total dry matter yield of native prairie grassland at the University of Alberta ranch at Kinsella, Alberta.

	Total dry matter yield - g. per sq. yard.				
	Number of applications of CCC at 3 lbs./a.				
	1	2	3	4	Mean.
CCC	58.1	69.2	70.0	79.4	69.4
Control	66.5	61.2	62.4	110.2	75.2

Data used - mean of 3 replicates; harvested on August 22nd 1961

differences between the yields of different untreated plots, these variations being of the same magnitude, or larger, than any effects that could have been attributed to CCC treatment. These observations suggest that, under field conditions in Alberta, CCC must be applied in relatively large doses to obtain measurable effects.

Table II. Effect of increasing CCC dose on the total dry matter yield of native prairie grassland at the University of Alberta ranch at Kinsella, Alberta.

Dry weight per sq. yd., in g., of:	Lbs./a. of CCC 0	$7\frac{1}{2}$	15	30	L.S.D. 5%	1%
Grass	26.0	36.0	41.7	31.3	8.1	11.7
Sagewort	28.9	22.3	23.5	28.0		
Golden rod	2.2	6.5	4.6	3.8		
Rest	5.9	5.3	7.0	4.3		
Total	63.0	70.1	76.8	67.4	12.7	-

Data used - mean of 3 spraying dates and of 3 replicates; harvested August 21st 1961

CCC applied at o, $7\frac{1}{2}$, 15 and 30 lbs./a. on the dry prairie grassland at Kinsella produced significant changes in the dry matter yield. Table II shows that the total dry matter yield was increased over that of the controls at all levels of CCC. Plots treated with CCC at 15 lbs./a. yielded 22 percent more than the controls, a difference significant at the 5 percent level. It should be emphasised that this increase appeared to be attributable to increased grass yield, rather than increases in any other component of the vegetation. Table II

shows this clearly; the yield of grass after application of CCC at 15 lbs./a. was 73 percent higher than that of the controls, and was significantly different at the 1 percent level. CCC treatment caused no significant changes in the dry matter production of the other species present; any observed differences were considered to be the result of random variation from plot to plot. Thus these results indicate that CCC is more effective on the grass and sedge component of the dry prairie grassland than on the broad leaved species present. Table III shows the effect of the different dates of spraying, and although these spanned a period of six weeks no statistically significant differences were recorded.

Table III. Interaction between date of spraying and CCC application on the dry matter yield of native prairie grassland at the University of Alberta ranch at Kinsella, Alberta.

		Date of spraying June 1st	June 22nd	July 12th
Grass dry weight per sq. yd., - g.	0	26.9	20.9	30.3
	CCC	36.9	36.2	34.1
Total dry weight per sq. yd., - g.	0	69.4	57.2	62.5
	CCC	72.7	72.2	67.4

Data used - for controls: mean of 3 replicates (3 plots)
for CCC-treated: mean of 3 CCC treatments and of 3 replicates (9 plots); harvested August 21st 1961

The results of the repeat spraying in 1962 of part of the above described experiment resembled those obtained one year earlier, as shown in Table IV. The magnitude of the effect

Table IV. Effect of increasing CCC dose on the total dry matter yield of native prairie grassland at the University of Alberta ranch at Kinsella, Alberta.

Dry weight per sq. yd., in g., of:	Lbs./a. of CCC 0	$7\frac{1}{2}$	15	30
Grass	47.2	99.8	115.8	47.0
Sagewort	126.0	133.3	99.8	137.2
Rest	10.0	27.2	18.4	12.0
Total	183.2	260.2	234.0	196.3

Data used - mean of 3 replicates; harvested August 15th 1962

was considerably greater than in the previous year; an increase of 145 percent over that of the controls was recorded for the yield of plots receiving 15 lbs./a. of CCC. It is possible that this was due to a carry over of some beneficial effect from the treatment in the previous year. A comparison of the results in Tables II and IV reveals that the general yield level was higher in 1962 than in 1961; this was probably due to a higher rainfall in 1962. It is clear that it was again the grass and sedge component of the vegetation which was affected to the greatest degree.

No significant differences in yield were recorded for the 1963 experiment at Kinsella. Table V shows the total dry matter yields for this experiment. CCC had no effect at 5 lbs./a., but at 10, 20 and 40 lbs./a. reductions in yield of 5, 7 and 17 percent respectively were produced; these, however, were not statistically significant differences. Any differences between different dates of spraying were again small and could not be considered as effects of date of CCC

Table V. Effects of CCC dosage and spraying date on the total dry matter yield of native dry prairie grassland at the University of Alberta ranch at Kinsella, Alberta.

		Date of spraying			Mean of 3 spraying dates
		May 15th	June 8th	June 27th	(= effect of CCC)
	0	1,384	1,862	1,774	1,673
	5	1,503	1,676	1,849	1,676
Lbs./a. of CCC.	10	1,521	1,590	1,621	1,585
	20	1,611	1,511	1,537	1,553
	40	1,363	1,294	1,488	1,382
Mean of CCC treated plots (= effect of spraying date).		1,500	1,518	1,597	-

Data used - dry weight in g. per 6½ sq. yds. (19x3 ft. swath). mean of 5 replicates; harvested September 4th 1963

Table VI. Effect of CCC on the dry matter production of cultivated brome grass at the University of Alberta farm at Ellerslie, Alberta.

		Treatment means
	0	3,259
	5	3,363
Lbs./a. of CCC.	10	3,253
	20	3,132
	40	2,880
L.S.D. at 5%		368

Data used - dry weight in g. per 6½ sq.yds. (20x3 ft. swath). mean of 5 replicates; harvested September 18th 1963

application. The results presented in Table VI show the dry matter yields for the brome experiment at Ellerslie. No effect was again recorded at 5 lbs./a. of CCC or at 10 lbs./a., with a reduction of 4 and 12 percent at 20 and 40 lbs./a. respectively, the latter being just significant at the 5 percent level.

The results of the last two experiments were at variance with those of the preceding ones. An increase in drought resistance of beans after treatment with CCC has already been described (17), and if this result can be extended to include grasses, then it is possible to explain the observed discrepancy between the results of the above described experiments. The 1961 and 1962 experiments were situated on a level area which had only about 4 to 5 inches of top-soil overlying a coarse gravelly sub-soil, which meant that the top-soil was dry and could dry out quickly after rainfall. The summer of 1961, in addition to this, was relatively dry in comparison with the subsequent years. In contrast with the 1961 and 1962 experiments at Kinsella the one conducted in 1963 was located in a slightly lower area having a considerably deeper top-soil; this would probably lead to the soil being wetter and drying out more slowly. The difference in response can thus be tentatively explained by assuming no differences developed in 1963 because moisture was not limiting, whereas during 1961 and 1962 there was s shortage of moisture which permitted the possible drought resistance effect of CCC to cause differences in yield to be produced. Recent reports indicate that treatment of wheat seedlings (37) and soybeans (34) with CCC increased their ability to tolerate higher

salt concentrations in comparison with that of untreated plants. As the Kinsella soil has a high salt concentration (137 lbs./a. of available potassium according to the 1961 soil analysis), it is possible that the observed yield differences could be attributed to this. In a dry year moisture evaporating from the soil surface would tend to increase the salt concentration in the upper layers of the soil, whereas rainfall in wetter years would tend to decrease, rather than increase, this salt concentration. If the effect of CCC on the salt tolerance of wheat seedlings is a general effect of CCC on grasses, then the magnitude of its effects on the yield at Kinsella could be expected to be greater in dry years than in wetter ones. Regardless of which tentative explanation could be correct, the lack of effect of CCC on brome at Ellerslie would not be suprising, as the soil was relatively deep and the rainfall adequate in 1963. It should be pointed out that the results of the two latter experiments were in accordance with those obtained for the greenhouse experiments. This aspect will be discussed again later.

EXPERIMENTAL II. GREENHOUSE EXPERIMENTS

Materials and methods

(a) General

Experiments described in this section were conducted in conventional heated greenhouses on the campus of the University of Alberta at Edmonton, except in a few instances when a controlled environment growth chamber was used. The greenhouse was maintained at a minimum temperature of 20°C.. During the winter the temperature remained approximately constant at this level, but during the summer, on sunny days, temperatures in excess of 35°C. were occasionally experienced. Supplementary lighting was given during the winter months to keep the day-length at 16 to 18 hours. Two-hundred watt incandescent bulbs spaced two feet apart and three feet above the pots were used for this purpose, giving a light intensity of approximately 500 ft-c. at foliage level. No attempt was made to control the humidity.

Plastic pots were used as the plant containers except in a few experiments in which beakers or specially constructed 'bottomless' pots were used. The shape and size of the pots varied with the type of experiment and the species being grown. A standard soil mixture of three parts Edmonton black loam soil to one part of coarse sand and one part of peat was used in the early experiments, but this was abandoned in favour of a mixture of two parts soil to one part sand for all the subsequent work. Cleaning of the roots of the plants grown in the soil:sand mixture, in preparation for the determination of root dry weights, proved to be considerably easier and more accurate in the absence of peat, although small losses of

root material still occurred. A one cm. layer of perlite was used in the base of the pots to assist drainage. Watering in all except a few experiments, was from shallow metal trays which were flooded with water as required, allowing the pots to soak up the necessary amount. This method avoided wetting the foliage and damage by flooding around the bases of small, relatively weak, grass seedlings. Elephant brand nitrogen fertilizer (analysis 33-0-0) with added RX-15 fertilizer (Garden Research Labs., Toronto, Ontario: analysis 15-30-15 of N-P-K plus trace elements) was supplied at a ratio of nine parts of nitrogen fertilizer to one part RX-15 at approximately six week intervals. The quantity used was calculated to supply the equivalent of a total of 200 lbs./a., and was applied with the water.

Application of the treatment solutions was by foliar spray for the majority of the experiments. This method was used in preference to soil drenches or related techniques since it seemed probable that if commercially useful effects were discovered, they would be of greater value if obtained using relatively low volume spray techniques rather than high volume soil drenches which had previously been found to be very effective (31,48,51,53,64). A specially designed cabinet sprayer was used to apply the treatment solutions. This consisted of a single nozzle boom which was mechanically propelled along the length of the cabinet. At 40 p.s.i. and with a type 650067 'Tee Jet' nozzle it delivered 13 gal./a. for one passage down the length of the cabinet. This volume was used for the early work, but when, as previously described, the strength of the CCC stock solution was lowered from 50

percent (w/v) to 11.8 percent (w/v) an increase in volume applied was again necessitated. This was effected by allowing the nozzle to pass over each pot three times, thus applying a total volume of 39 gal./a.. Tween 20, at 0.1 percent, was used as a wetter throughout the greenhouse experiments.

(b) Effects of CCC on regrowth of grasses after clipping.

An investigation of the effects of CCC on the recovery of Kentucky bluegrass (*Poa pratensis* L.) and creeping red fescue (*Festuca rubra* L.) from clipping was carried out in the greenhouse. Rectangular 4x5 inch plastic pots 4 inches deep were used and peat was included in the soil mix, as no attempt was made to obtain dry weight data for the roots. Spraying was carried out 6 weeks after sowing, the plants then being allowed to grow for a further 6 weeks, after which they were clipped and the samples thus obtained were dried and weighed. The plants were allowed to grow for 6 weeks after this and were then clipped again, so that the effects of CCC, if any, on regrowth could be ascertained. A second similar experiment was conducted using fescue only.

(c) Studies on the morphology of CCC treated grasses, including root and shoot development and responses of treated plants to different light intensities and their resistance to drought.

Experiments to investigate the effects of CCC on the morphological development of three grass species were all carried out using similar methods. Caryopses were sown in excess into pots. After two to three weeks of growth the seedlings were thinned to one per pot. Preliminary experi-

ments using large numbers of plants per pot gave extremely variable results, and because of the large number of plants involved, measurements of morphological characteristics were difficult. By using one plant per pot and increasing the number of replications from 3 or 4 to 10 to 12 (to compensate for the smaller individual sample size), it was possible to increase the accuracy considerably and to make the measurement of morphological characters relatively easy. At the time of spraying, which varied somewhat depending on the objectives of the experiment and the species being used, the plant height was measured (as distance from the soil surface to the tip of the tallest leaf), and the number of fully expanded leaves and the number of tillers and/or lateral shoots, including those from rhizomes, were determined. These measurements and counts were made weekly from the time of spraying until the experiment was terminated. Harvesting consisted of removing the plant and the soil from the pot, washing as much soil as possible from the roots and storing the plant in a sealed polyethylene bag kept at about $3^{o}C$. until further measurements could be made. Plants kept by this method of storage were found to deteriorate very slowly, remaining green and not showing signs of rotting for several weeks. Measurements of rhizome length were made (if rhizomes were produced) and the number of underground stem apices was determined. Each plant, consisting of separate samples of tops, roots and rhizomes, was dried in an electric oven at approximately $80^{o}C$. and weighed.

Kentucky bluegrass received most attention using the above described techniques. Experiments were conducted to ascertain the effects of CCC in both the winter and the summer

and to study the effect that plant age at the time of spraying might have on the development of differences caused by CCC. Investigations were also carried out to determine the effect of CCC on the morphological development of creeping red fescue and brome grass; leaves from plants in the latter experiment were used for chlorophyll analyses. Wheat (var. Thatcher), oats (var. Victory) and barley (var. Montcalm) were grown in an experiment in an attempt to confirm previously described results and also to compare the degree to which the three cereal species were affected by CCC.

Plants that were used for the physiological studies (described later) were also measured to assess effects of CCC on morphological characteristics. These plants were grown in the dark in a germinating cabinet maintained at 20°C.. In order that bacterial and fungal infection could be minimized, steam sterilization at 110°C. for one hour of the 1,000 ml. beakers and vermiculite growing medium was done prior to sowing. Corn 'seeds' (hybrid var. Wheatland) were sown onto 400 mls. of vermiculite and were covered by a further 100 mls.; 310 mls. of treatment solution were found to be sufficient to moisten all the vermiculite thoroughly. Wheat 'seeds' (var. Thatcher) were sown onto 250 mls. of vermiculite and covered with 100 mls. more; 210 mls. of treatment solution were found to be adequate for this experiment. Corn seedlings were grown for 14 days and wheat for 10 or 11 days, at which time they were harvested for use in the physiological investigations; prior to these, measurements of height were made for both species, plant volume was measured for corn, root length was measured for wheat and dry weights

were obtained for both species after freeze-drying on a Virtis freeze-drier.

Previous work had indicated that CCC affects root development (8,30,32); because of this and because of difficulties experienced in obtaining reliable results for the yield of root dry matter in pots, a further experiment was conducted in an attempt to obtain useful data on this aspect. A special 'bottomless' pot was constructed for this experiment. Black polyethylene piping, 4¼ inches in diameter, was cut into 4-inch lengths. Pieces of #16 gauge galvanized wire were sharpened and heated at one end and then inserted, by melting the polyethylene, across one end of each pipe section to form an open mesh screen of about 0.75-1.50 cm. squares. Four wire ends, one at each quarter, were left protruding about 2 cms; the pots were then suspended by these wires about 0.5-1.0 cm. above water contained in two metal tanks. Roots were thus able to grow down into the water below and could be measured without difficulty. Light entering the aerated water was minimized by placing the pots through holes cut in a sheet of roof felt. Soil was retained in the pots by a layer of coarse peat moss above the screen. After seeding, the pots were placed and maintained over the water until spraying, at which time measurements of root and top length were made. Routine measurements of these were made once every subsequent two weeks and at the first clipping 6 weeks after spraying. The advantage of the 'bottomless' pot was that it allowed the clipping of root growth as well as the tops, and also study of their subsequent regrowth. Data were obtained for the dry matter yield of both the tops and the roots at the first

clipping. Further measurements on the root and shoot regrowth were made at 2,4 and 6 weeks after the first clipping; a second clipping being made at the latter date.

Two experiments were conducted to investigate the possible interaction between CCC and light intensity. Kentucky bluegrass plants, prepared and treated in exactly the same manner as those used in the previously described morphological studies, were grown in a controlled environment growth chamber (Coldstream Refrigerator Mfg. Ltd., Winnipeg, Manitoba. - model 200/300 modified). A wooden frame was constructed inside this growth chamber to divide it into four sections. The light intensity in each section was modified by filters consisting of multiple layers of glass and cheese-cloth, arranged to allow intensities of the maximum produced by the lights in the growth chamber, then approximately 3/4, 1/2 and 1/4 of this respectively. Daylength was set at 18 hours and the temperature at a constant $20^{\circ}C$.. Measurements and sampling procedures were similar to those for the morphology experiments.

An experiment was set up using methods similar to those of Halevy and Kessler (17) for beans, to test the effect of CCC on the drought resistance of creeping red fescue. 'Seeds' were sown into glazed two gallon earthenware pots containing equal quantities of soil. After two weeks growth the seedlings were thinned to 10 per pot and after $4\frac{1}{2}$ weeks they were sprayed. Two weeks after spraying, the pots were placed in a deep water bath and allowed to reach saturation point. They were then allowed to drain for two hours, after which time the holes at the base were sealed. Four weeks after

the soaking, during which no further water was added to the pots, data were obtained for the yield of tops and root dry matter, and for the amount of water remaining in the soil.

With the objective of avoiding confusion, some additional specific points of procedure peculiar to the various experiments are included with the ensuing presentation of the results.

Results

(1) General observations

The effect of CCC application could be seen after approximately one week in most experiments, and in a few cases sooner than that. Brome grass grown from rhizomes appeared to be fairly susceptible to high doses of CCC, considerable scorch occurring at 20 and 40 lbs./a. of CCC, and in a few instances death of the treated plants occurred. These were the only instances of plants dying after treatment with CCC recorded during the work presented here. Apparently this phytotoxicity was due to the peculiar nature of the affected plants; this will be discussed later.

Patchy chlorosis of the leaves was one of the most obvious early responses of plants sprayed with CCC. Species with wide leaves, such as barley and brome, showed this effect most clearly, and it could scarcely be seen on creeping red fescue plants with their narrow, rolled leaves. Fig.1. shows the appearance of this chlorosis on leaves of barley plants that had received 5 lbs./a. of CCC. Lindstrom and Tolbert (31) also reported this symptom developing after

Fig. 1. Patchy chlorosis on the leaves of barley plants treated with 5 lbs./a. of CCC; 10 days after spraying.

spraying CCC solutions onto plants, but they also reported that the leaves recovered. The present author observed that the chlorotic areas remained, but that leaves on which this effect was produced died as the plants grew older and larger. New, and thus unsprayed, leaves were perfectly healthy in all species investigated and almost invariably appeared to be a darker green than those of the control plants. Therefore, as the leaves on which the chlorotic areas developed had died, the plants regained a healthy appearance. Darker green foliage of plants treated with CCC was usually apparent for the duration of all experiments, except those conducted in the dark.

Reduction in height usually became obvious one and a half to two weeks after spraying. This effect was most noticeable for species normally producing long internodes, such as wheat, barley and brome. The effect of CCC on the height of creeping red fescue, although measurable, was in most cases

not readily visible. These results substantiate the general findings of other workers to the effect that CCC treated plants are shorter and a darker green than the controls.

(2) Effect of CCC on the recovery of Kentucky bluegrass and creeping red fescue from clipping.

The primary yields of clipped foliage from Kentucky bluegrass and creeping red fescue were not affected to any significant degree as a result of spraying the plants with CCC. This is shown in Table VII in the column for first clipping six weeks after spray treatment. It can also be seen that after allowing the plants to grow for an additional six weeks, the yields were greater for those plants that had originally

Table VII. Effect of CCC on the dry matter yields of Kentucky bluegrass and creeping red fescue and on their recovery from clipping.

		Kentucky bluegrass		Creeping red fescue	
		1st clipping	2nd clipping	1st clipping	2nd clipping
Lbs./a. of CCC.	0	4.53	2.83	3.64	2.47
	2½	4.64	3.37	3.86	3.39
	5	4.56	3.01	3.24	2.44
	10	4.20	3.26	3.03	2.63
	20	4.55	3.47	3.37	3.21
	40	3.87	3.22	2.88	2.90
L.S.D. -	5%		-		0.57
	1%		-		0.78

Data used - dry matter yield per pot, in g.; mean of 6 replicates. 1st clipping 6 weeks after spraying, 2nd clipping 12 weeks after spraying.

Table VIII. Effect of CCC on the dry matter yield of creeping red fescue and its recovery from clipping - repeat experiment.

		1st clipping	2nd clipping
Lbs./a. of CCC.	0	3.07	7.61
	$2\frac{1}{2}$	3.23	7.33
	10	2.92	7.10
	40	2.69	7.33

Data used - dry matter yield per pot, in g.; mean of 6 replicates

been sprayed with CCC than those which had not (Table VII - 2nd clipping). The increases in yield that occurred for CCC-treated Kentucky blue grass in comparison with that of the controls was not significantly different, probably because of a high level of variation. The results for the creeping red fescue, although also showing a high level of variation, showed significant differences at the 1 percent level, indicating that CCC had increased the ability of the plants to recover from clipping.

A repeat experiment, using methods similar to those of the first one, was carried out using creeping red fescue only. The results of this experiment are presented in Table VIII; a similar trend for a slight increase in yield over that of the controls at the low rates of CCC and a reduction at higher rates was again recorded for the initial clipping, although these differences were not statistically significant. The yields obtained at the second clipping, although generally higher than those obtained for the first experiment due to a longer interval between the two clippings, did not show any

trend for increases resulting from treatment of the plants with CCC, but rather a slight, non-significant, decrease.

The results of these two experiments tend to confirm the findings of the latter field experiment at Kinsella, indicating that although CCC may increase the ability of plants to recover from clipping, it does not alter the primary yield of fescue to any great degree and that there is a tendency for reduced yield following the higher rates of CCC application. This latter effect will be discussed when considering the effects of this chemical on the morphological development of creeping red fescue.

(3) Effects of CCC on the morphological development of of Kentucky bluegrass.

The first experiment to investigate the effects of CCC on the morphological development of Kentucky bluegrass was carried out during the winter, and was terminated at the relatively short time of five weeks after spraying. Measurements made at this time showed that increasing doses of CCC produced a trend for decreased plant height, decreased rhizome production (as rhizome length per plant), increased tillering and increased leaf production (Fig. 2.). The differences caused by CCC for all four characteristics were significant at the 1 percent level. Plant height was measured once every week from the time of spraying to the end of the experiment; the results presented in Fig. 3. show that the effect of CCC on plant height developed rapidly after spraying. Treated plants continued to elongate at a rate only slightly lower than that of the controls for the first week after spraying,

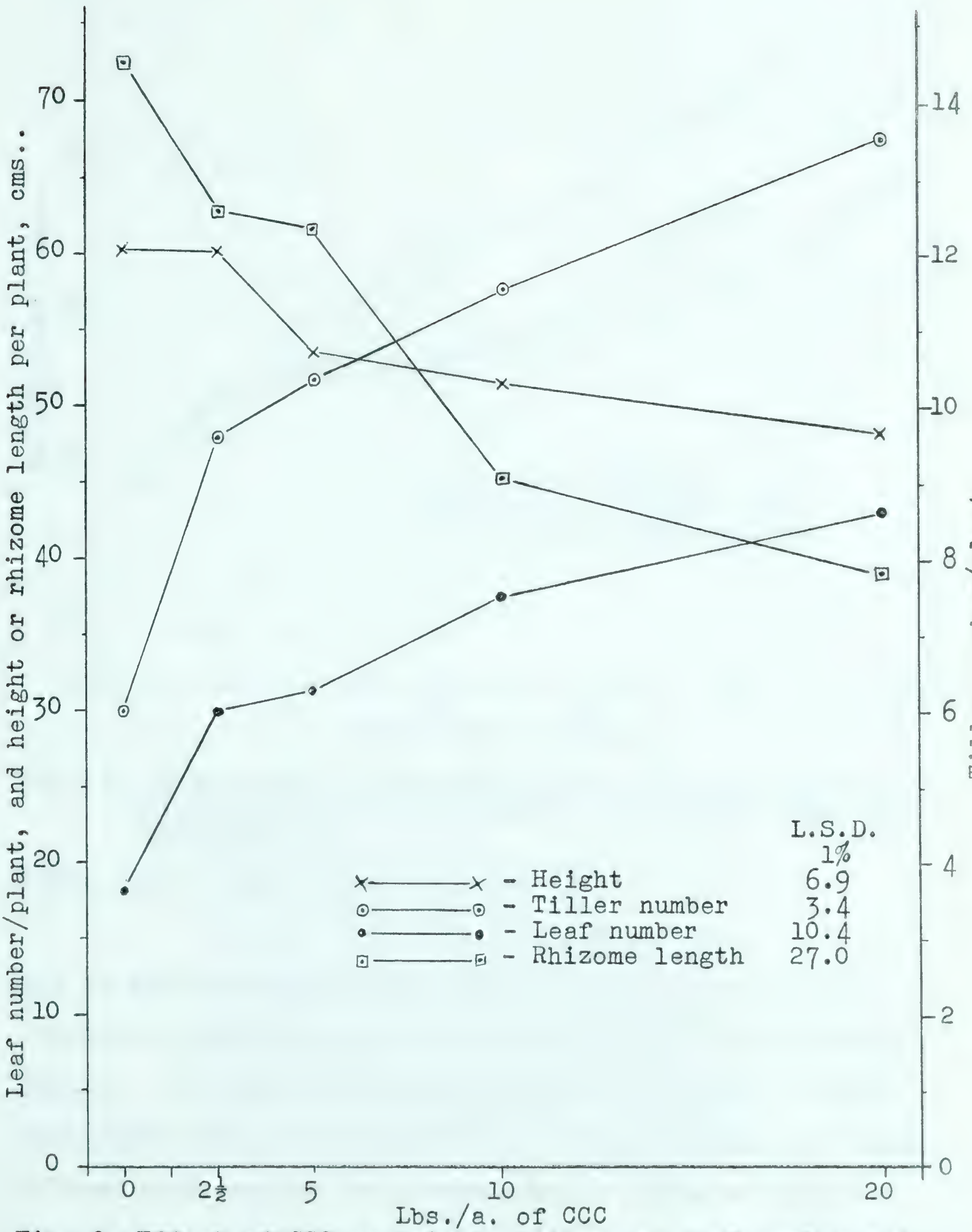


Fig. 2. Effect of CCC on height, tiller and leaf number and rhizome length of winter grown Kentucky bluegrass.

(Data used - mean of 13 replicates; 5 weeks after spraying).

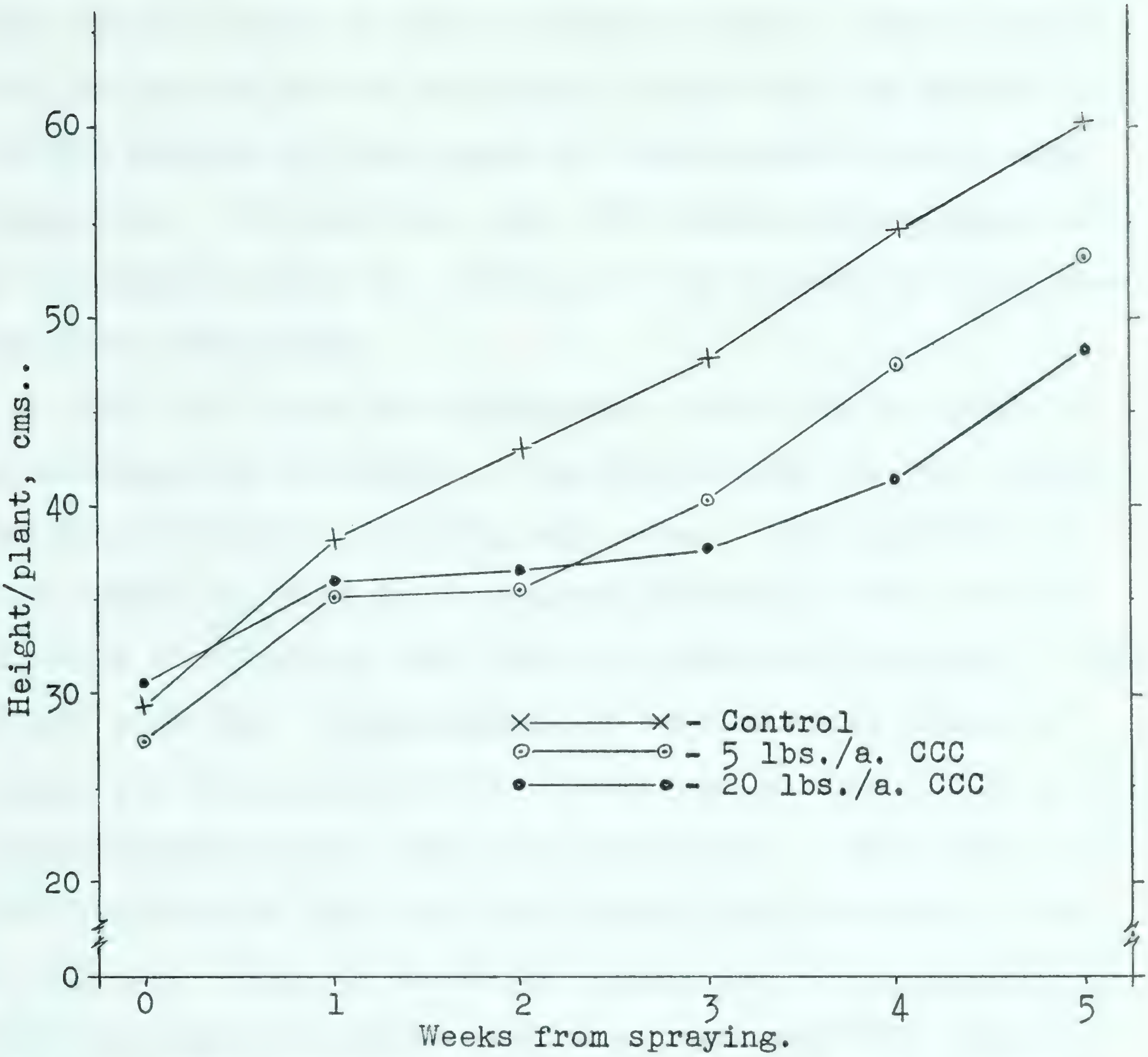


Fig. 3. Development of the effect of CCC on winter grown Kentucky bluegrass during the first five weeks after spraying.

(Data used - mean of 13 replicates)

but in the subsequent week their rate of elongation was relatively slight in comparison with that of the untreated plants. At three weeks after spraying the plants treated with light doses of CCC started to elongate again, but those treated with heavier doses continued to elongate slowly. Towards the end of the experiment, however, all treated plants were elongating at a rate equal to or greater than that of the

controls. No similar data could be obtained for the development of the effect of CCC on rhizome length. Measurements made at the end of the experiment showed that the effect of CCC was greater on this aspect of plant growth than on stem elongation. It was clear that CCC inhibits the elongation of underground stems in addition to its effects on those growing above the ground.

The leaf number was determined weekly and was found to be increased by treatment of the plants with CCC, the trend for this increasing steadily with time. This increase in leaf number appeared to be related directly to the observed increase in tillering that resulted after the treatment of the plants with CCC. Calculations to determine the number of leaves per tiller showed that there were no significant differences between treated and untreated plants. This close correlation between leaf and tiller number can be clearly seen in Fig. 2.. Fig. 4. shows the appearance of representative Kentucky bluegrass plants from each treatment with regard to

Fig. 4. Effect of CCC on winter grown Kentucky bluegrass, at four weeks after spraying.

the above described visible morphological effects.

Plants treated with CCC all produced dry matter yields that were significantly higher than those of the controls, but there were no significant differences between the yields of plants receiving the four different rates of CCC, as shown in Fig. 5.. The increase produced by 2½ lbs./a. of CCC was almost equal to that produced by 20 lbs./a.. This effect was probably attributable to the interaction between decreased stem length and the increased tiller and leaf production. CCC applied at 2½ lbs./a. reduced plant height slightly, but increased the tillering and

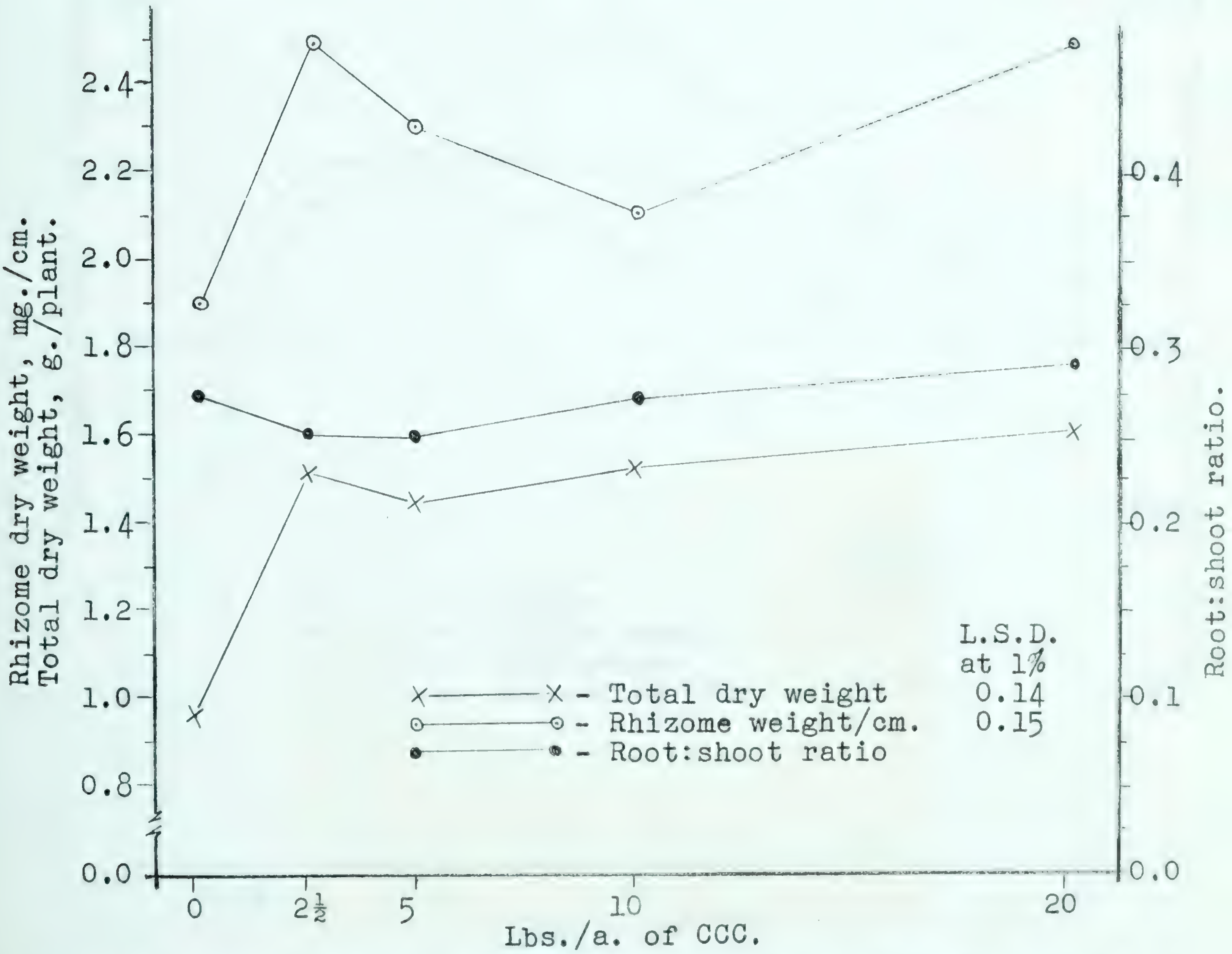


Fig. 5. Effect of CCC on total dry dry matter yield, dry weight of rhizome per cm. and root:shoot ratio of winter grown Kentucky blue grass.
(Data used - mean of 13 replicates; harvested 5 weeks after spraying

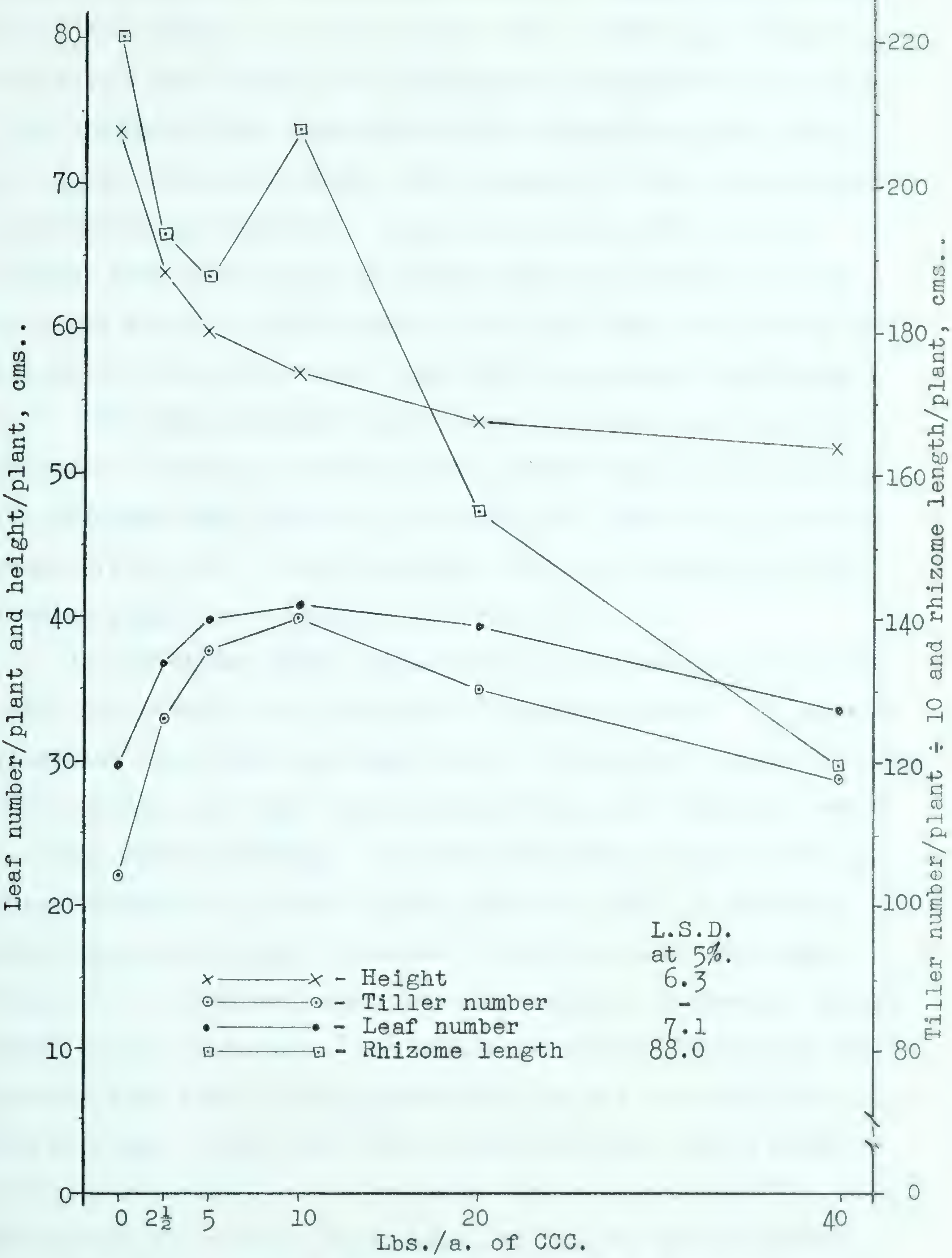


Fig. 6. Effect of CCC on height, tiller and leaf number and rhizome length of summer grown Kentucky bluegrass. (Data used - mean of 12 replicates; 6 weeks after spraying except for 10 weeks after spraying for rhizome length).

leaf production to a considerable extent, leading to increased total dry weight. At each higher dose of CCC any increase in dry weight that might have resulted from increased tiller and leaf production was compensated for by decreased stem length; the total dry matter yields thus remained at the approximately constant level observed. Data presented in Fig. 5. also suggest that the root:shoot ratios were not affected by CCC, but this result is questionable since peat was still being used in the soil mix at the time when this experiment was carried out. The data obtained for rhizome dry weight per cm., although not showing a smooth trend, showed that it was greater for rhizomes from treated plants than for those from untreated plants (Fig. 5.). This indicated that the rhizomes on CCC-treated plants were shorter, but thicker.

An experiment using methods similar to the one described above was carried out during the following summer. It closely resembled the first one except that a rate of 40 lbs./a. of CCC was included, and that the experiment was not terminated until 10 weeks after spraying. A trend for reduced height with increasing doses of CCC was again observed, being of much the same magnitude as that recorded for the previous experiment (Fig. 6.). Rhizome length was also reduced, although a smooth trend was not obtained; the effect on rhizome length was again greater than that on stem elongation, as can be seen in Fig. 6.. The increase in leaf and tiller production was not as great as had been recorded for the previous winter experiment (Fig. 6.), and unlike the earlier experiment, 20 lbs./a. of CCC caused less effect than 10 lbs./a., and 40 lbs./a. had reduced growth to a level where no change in tillering or leaf production

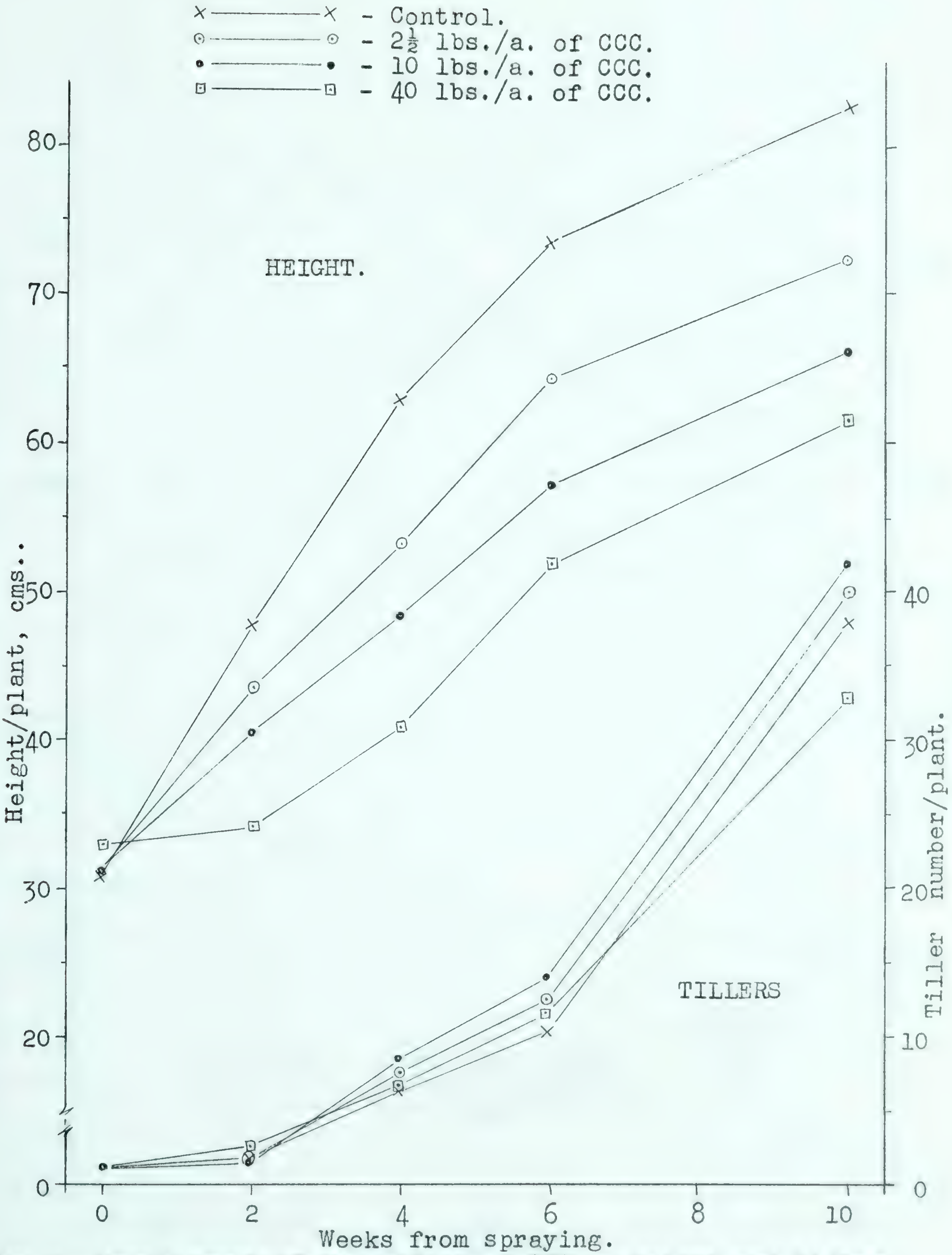


Fig. 7. Development of the effect of CCC on height and tiller number of summer grown Kentucky bluegrass.

(Data used - mean of 12 replicates).

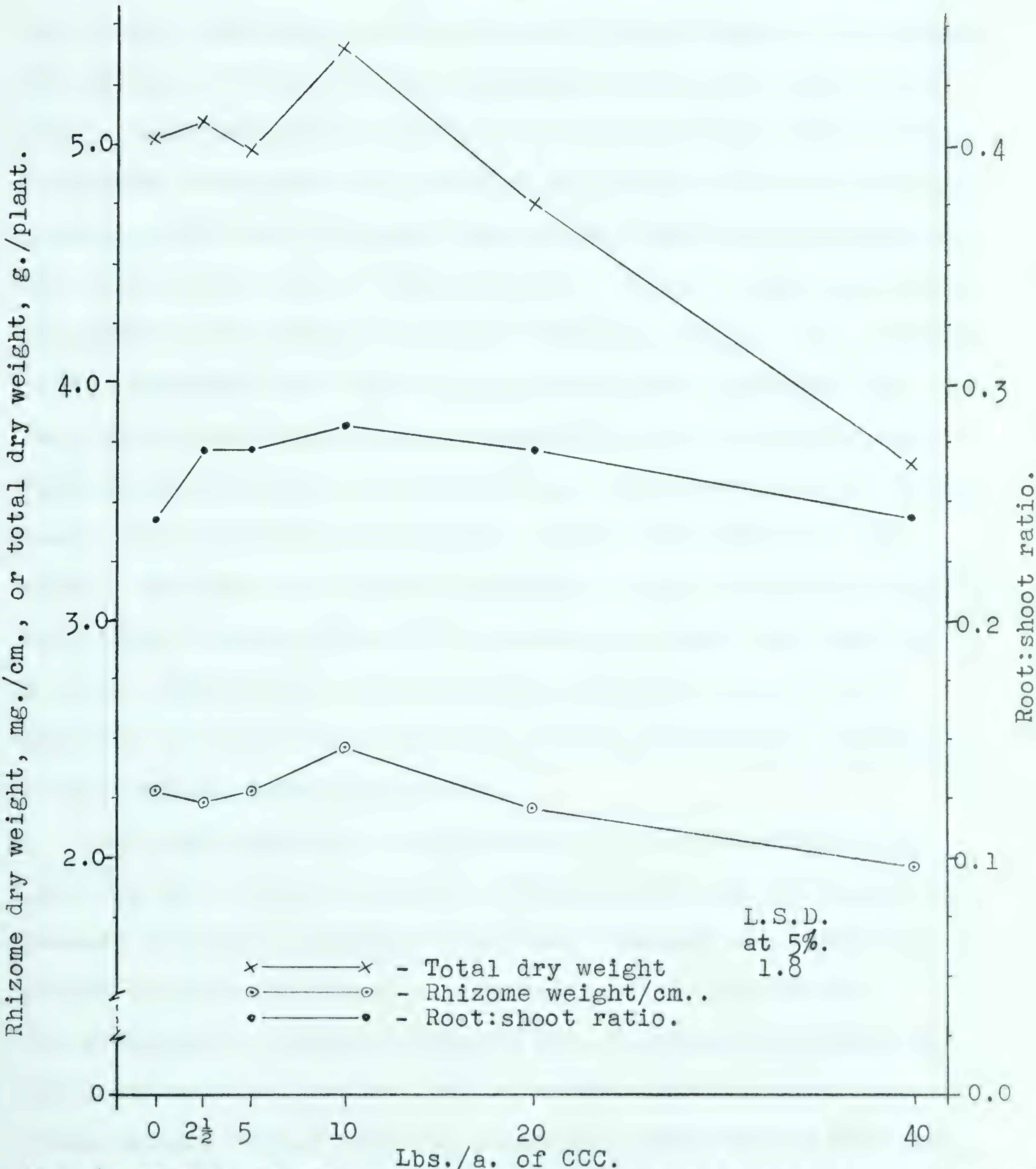


Fig. 8. Effect of CCC on total dry matter yield, rhizome dry weight per cm. and root:shoot ratio of summer grown Kentucky bluegrass.
(Data used - mean of 12 replicates; harvested 10 weeks after spraying).

took place, in comparison with that of untreated plants.

The data obtained for height and tiller number from weekly measurements are shown in Fig. 7.. No attempt was made to include leaf number data because, as can be seen from Fig. 6., leaf number was again related directly to the number of tillers. The effect of CCC on height developed rapidly, but only those plants that received 40 lbs./a. of CCC showed any severe check in growth after spraying, those of all other treatments showing gradual reductions in growth rate which could be correlated to the size of the dose of CCC received. Fig. 7. also shows that the maximum differences in height between treated and untreated plants occurred about four to six weeks after spraying, and that no further increase in the magnitude of the effect developed before the end of the experiment, as plants from all treatments then appeared to be growing at an approximately equal rate. The data for tiller production, also presented in Fig. 7., show that treatment with CCC produced only small and non-significant effects, and that the rate of 40 lbs./a. of CCC appeared to be detrimental to the growth of Kentucky bluegrass, as measured by tiller production.

The data obtained for dry matter yield also demonstrate that the dose of 40 lbs./a. of CCC was sufficient to cause suppressed growth in Kentucky bluegrass, although the plants appeared healthy throughout the duration of the experiment. The graphically presented data in Fig. 8. show this growth inhibition and also that 20 lbs./a. of CCC caused a slight decrease in dry matter yield in comparison with that of the controls. Only small and inconsistent increases were recorded for the lower levels of CCC. The root:shoot ratios were not

altered by treating the plants with CCC and only small, non-significant changes were recorded for the dry weight of rhizome per cm..

The effect of CCC on height and rhizome length per plant was of approximately the same magnitude for both of the experiments just described, but although the other morphological characteristics that were measured showed basically similar effects in the second experiment these were not so large as those for the previous experiment; this was especially apparent in regard to tiller production, dry matter yield and rhizome dry weight per cm.. It should be emphasised that the first experiment was carried out between January and March and that the second was from June to October. Other workers (21,53) have suggested that there might be an interaction between the effect of CCC and light intensity. It would appear from the results of the two experiments just described that a similar interaction may also occur for Kentucky bluegrass. Tolbert (53) reported almost exactly the same change in the effect of CCC when investigating its effects on winter and summer grown wheat plants.

In an attempt to obtain further evidence concerning this apparent difference in the effect of CCC between winter and summer, the winter experiment on Kentucky bluegrass was repeated. The rate of 40 lbs./a. was omitted because this dosage in the previous experiment appeared to be considerably in excess of the optimum. This experiment was terminated at eight weeks after spraying as the magnitude of the effect of CCC did not appear to change after this time in the previous experiment. The effects of CCC on height, rhizome length per

plant, tiller production and leaf number per plant followed patterns similar to those described for the two previous experiments, and the data obtained are graphically presented in Fig. 9.. The effects on height and rhizome length per

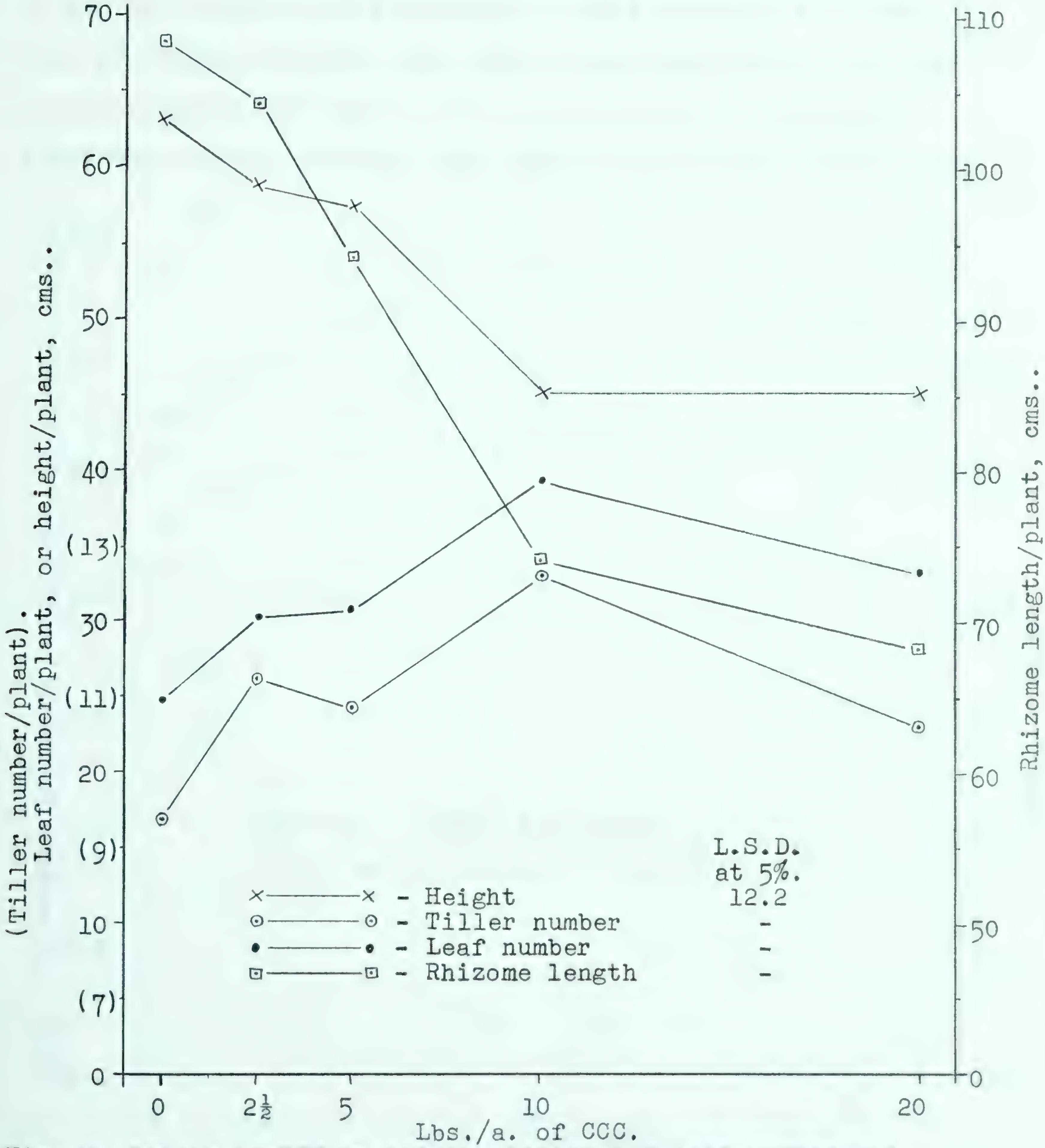


Fig. 9. Effect of CCC on height, tiller and leaf number and rhizome length of winter grown Kentucky blue grass - repeat experiment.
(Data used - mean of 12 replicates; harvested 8 weeks after spraying).

plant confirmed those previously described, and measurements made on height throughout the experiment showed that the pattern of development of this effect was also similar to that reported for the two earlier experiments. The magnitude of the effect of CCC on tillering, and thus also on the apparently related leaf production, was not as large as had been recorded for the previous experiment carried out in the winter. The differences were more consistent, and apparently somewhat larger, than

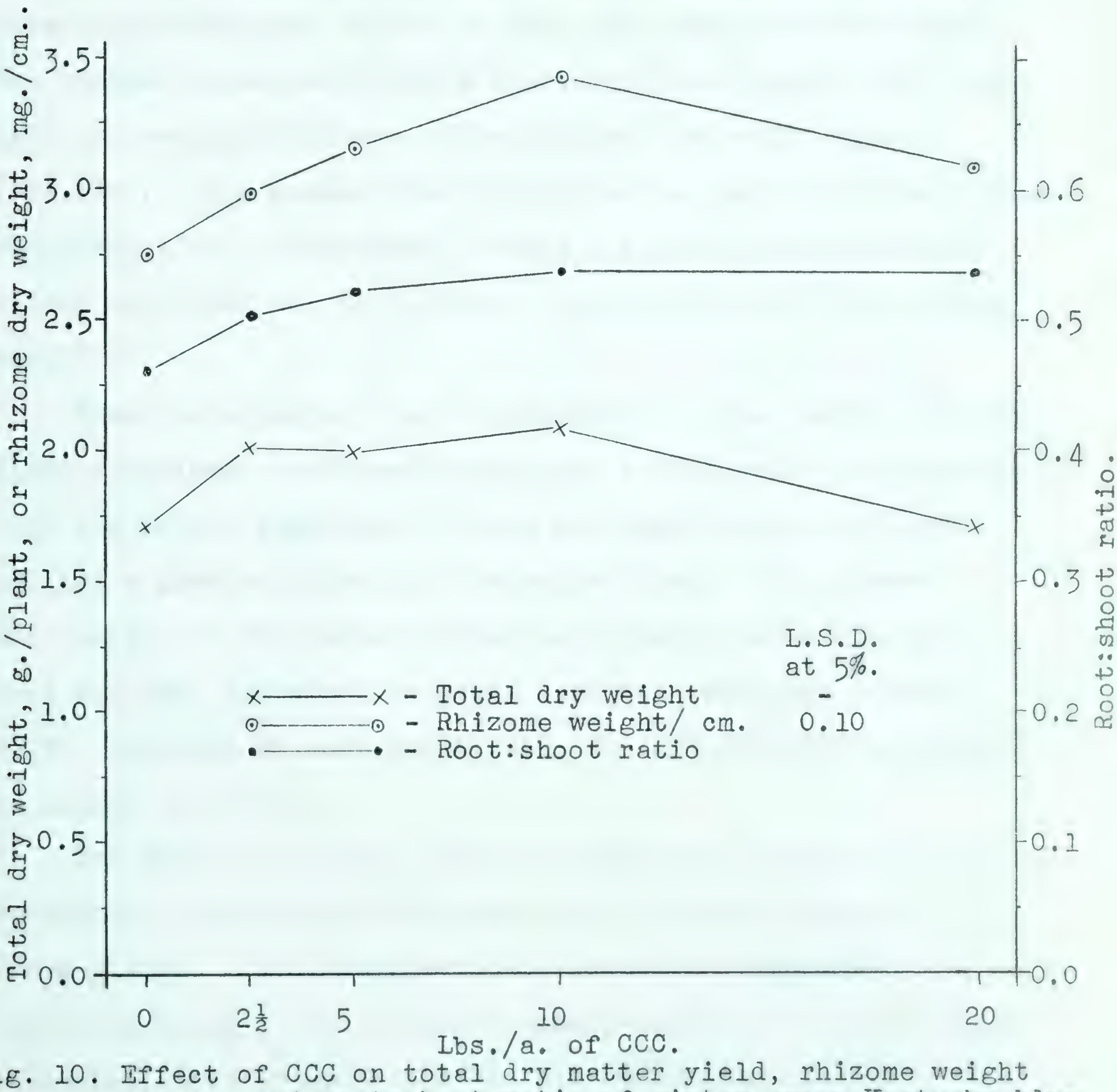


Fig. 10. Effect of CCC on total dry matter yield, rhizome weight per cm. and root:shoot ratio of winter grown Kentucky bluegrass - repeat experiment.
(Data used - mean of 12 replicates; harvested 8 weeks after spraying).

those recorded for the summer experiment, but were, however, not statistically significant. The dry matter yield was increased over that of the controls at all levels of CCC (Fig. 10.), but the differences were not statistically significant. This effect was not as large as that recorded for the first winter experiment, but was considerably greater than that obtained in the experiment conducted in the summer. The root: shoot ratios recorded for the previous two experiments had not shown any consistent effect of CCC, but those calculated for the present experiment showed a moderate, but consistent, rise with increasing CCC dose. The rhizome dry weight per cm. (Fig. 10.), in a manner exactly similar to that in evidence from the first winter experiment, showed a highly significant increase over that of the controls after treatment of the plants with CCC.

Thus, although an exact duplication of the results of the first experiment conducted during the winter, was not obtained from the repeat experiment, there was again indication that CCC had a greater effect in the winter than in the summer. The results of the latter winter experiment, using the soil/sand mix that contained no peat, also supported the reported (8,59) increase in root:shoot ratio brought about by treatment of plants with CCC.

The final experiment involving Kentucky bluegrass was an attempt to investigate the effects of CCC when applied to young plants. All the previously described experiments involved spraying at the stage of growth when tillering had just commenced, usually about five to six weeks after sowing. The plants in the present experiment were sprayed at about three

and a half weeks after sowing, before any tillers had emerged and when the seedlings were only just over 10 cms. tall, in comparison with the 25 to 30 cms. of the plants used in the other experiments. The 40 lbs./a. rate of CCC was again in-

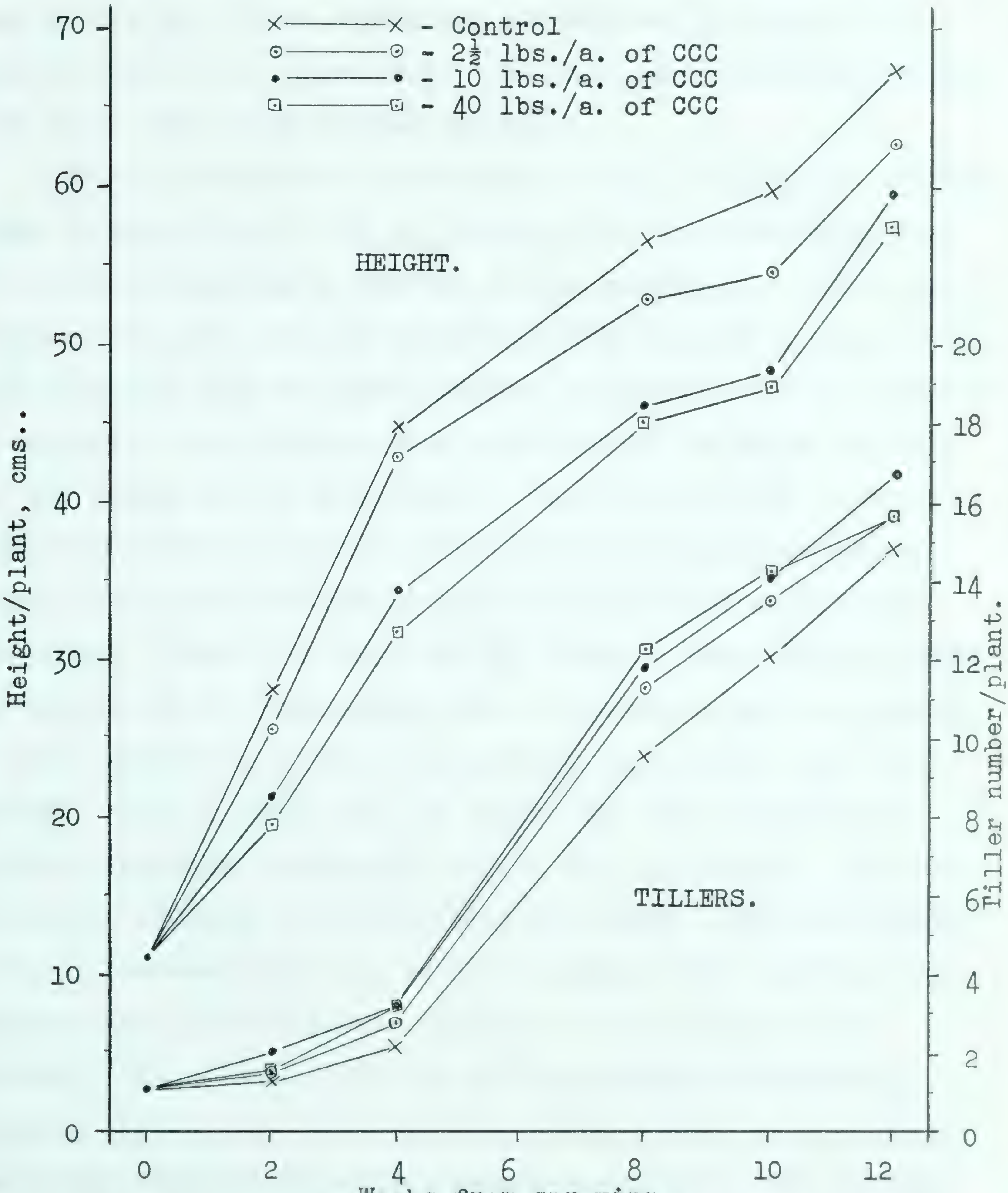


Fig. 11. Development of the effect of CCC on height and tiller number of Kentucky bluegrass, sprayed at an early stage (prior to tiller emergence).
(Data used - mean of 12 replicates)

cluded so that the effects of this extreme dose could be assessed on the young plants. It should be pointed out that this experiment was carried out during the summer and early fall. It was continued for 12 weeks after spraying so that the plant age at the end of the experiment was similar to that at the end of the earlier experiments, and thus made allowance for the two and a half weeks earlier spraying.

The data presented graphically in Fig. 11. show the development of the effect of CCC on height and tiller production from the time of spraying to the end of the experiment. It is of interest to note that the relatively high dose of 40 lbs./a. of CCC proved to have no lethal effects on these plants of Kentucky bluegrass; those treated with this rate of CCC being healthy at all stages of the experiment. The effect of CCC on plant height followed the pattern observed for the previous experiments, being at a maximum at about four to eight weeks after spraying. There did appear to be, however, some loss of effect by the end of the experiment with all treated plants growing at a rate faster than that of the controls during the last two weeks. This apparent loss of effect was also observed for tiller production towards the end of the experiment. The CCC treatment appeared to induce early tillering; untreated plants did not commence tillering until two weeks after spraying time, whereas many treated plants produced a tiller during that period. The effect of CCC on tiller production attained a maximum eight weeks after spraying, after which, as previously mentioned, some of the effect seemed to be lost. The differences produced after treatment with CCC were significant at two, four and eight weeks after spraying, but these signifi-

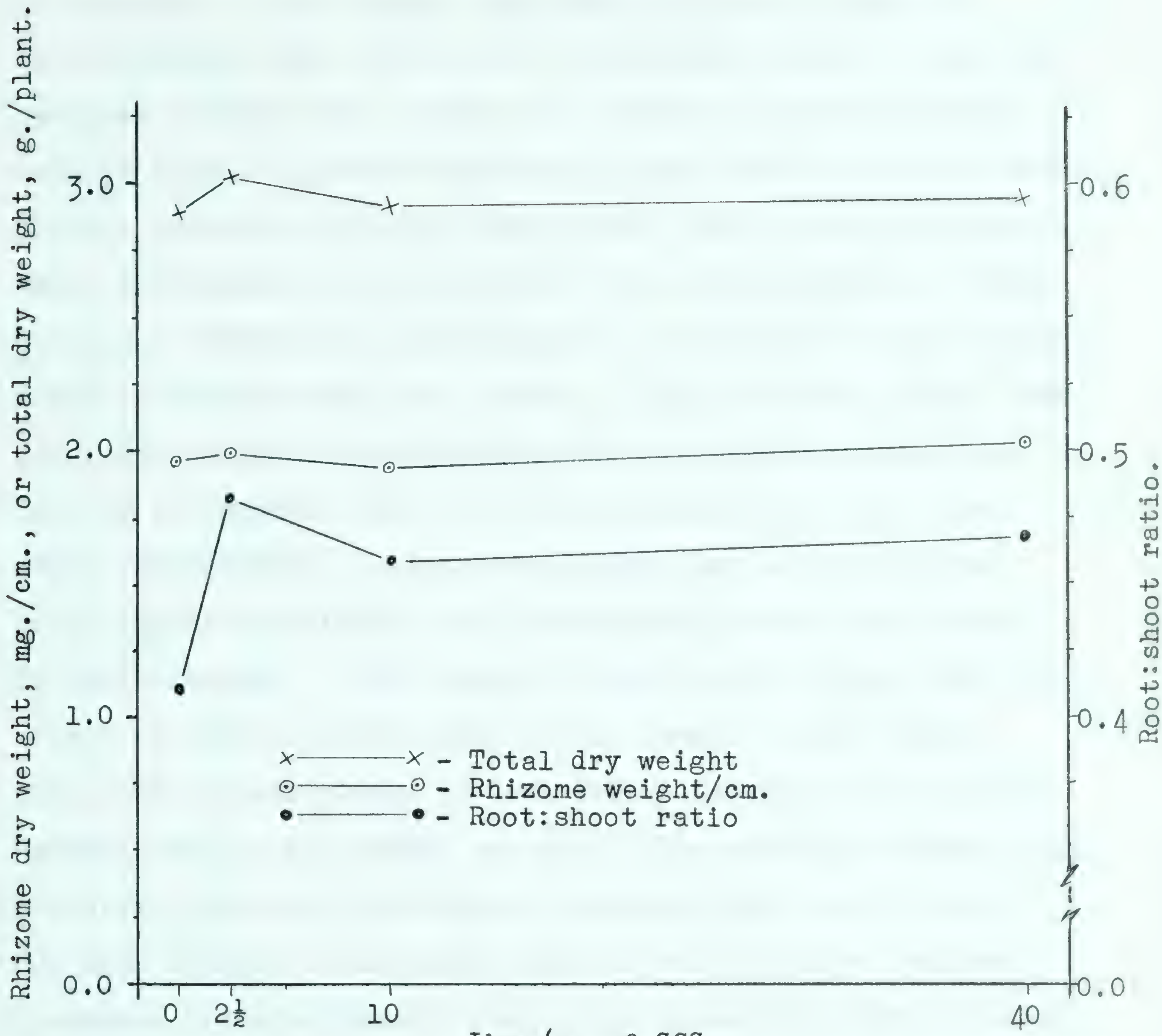


Fig. 12. Effect of CCC on total dry matter yield, rhizome weight per cm. and root:shoot ratio of summer grown Kentucky bluegrass, sprayed at an early stage (prior to emergence of tillers)
(Data used - mean of 12 replicates; harvested 12 weeks after spraying).

cant differences had been lost at the last two measuring dates. Data for leaf number, although not presented, followed a pattern similar to that for tillering. Rhizome lengths measured at the end of the experiment confirmed those obtained for the previous experiments.

The dry matter yields did not appear to have been affected by treatment of the plants with CCC, as shown in Fig. 12.. It is possible that differences had existed earlier in the experiment, but that the tendency for growth of treated and untreated plants to become equalized by the end of the experiment, already observed for height and tiller production, had reduced these differences to the low level that was recorded, or that the small differences produced were a result of the experiment being conducted during the summer. The root:shoot ratio, however, was considerably increased for all plants treated with CCC, in accordance with the results obtained for the second winter experiment. Rhizome dry weight per cm., as in the prior summer experiment, did not appear to have been altered by CCC treatment. This supplies additional evidence that the effect of CCC is not as great in the summer as the winter, since CCC-treated plants in both winter experiments showed increased rhizome dry weight per cm.. The results obtained from the above described experiment indicated that the effects of CCC when applied at an early stage in the growth of Kentucky bluegrass are considerable during the subsequent developmental growth, but that they tend to decrease as the plants reach maturity.

(4) Effects of CCC on the morphological development of creeping red fescue.

The procedure in a single experiment to investigate the effects of CCC during the spring and early summer on some of the morphological characteristics of creeping red fescue was

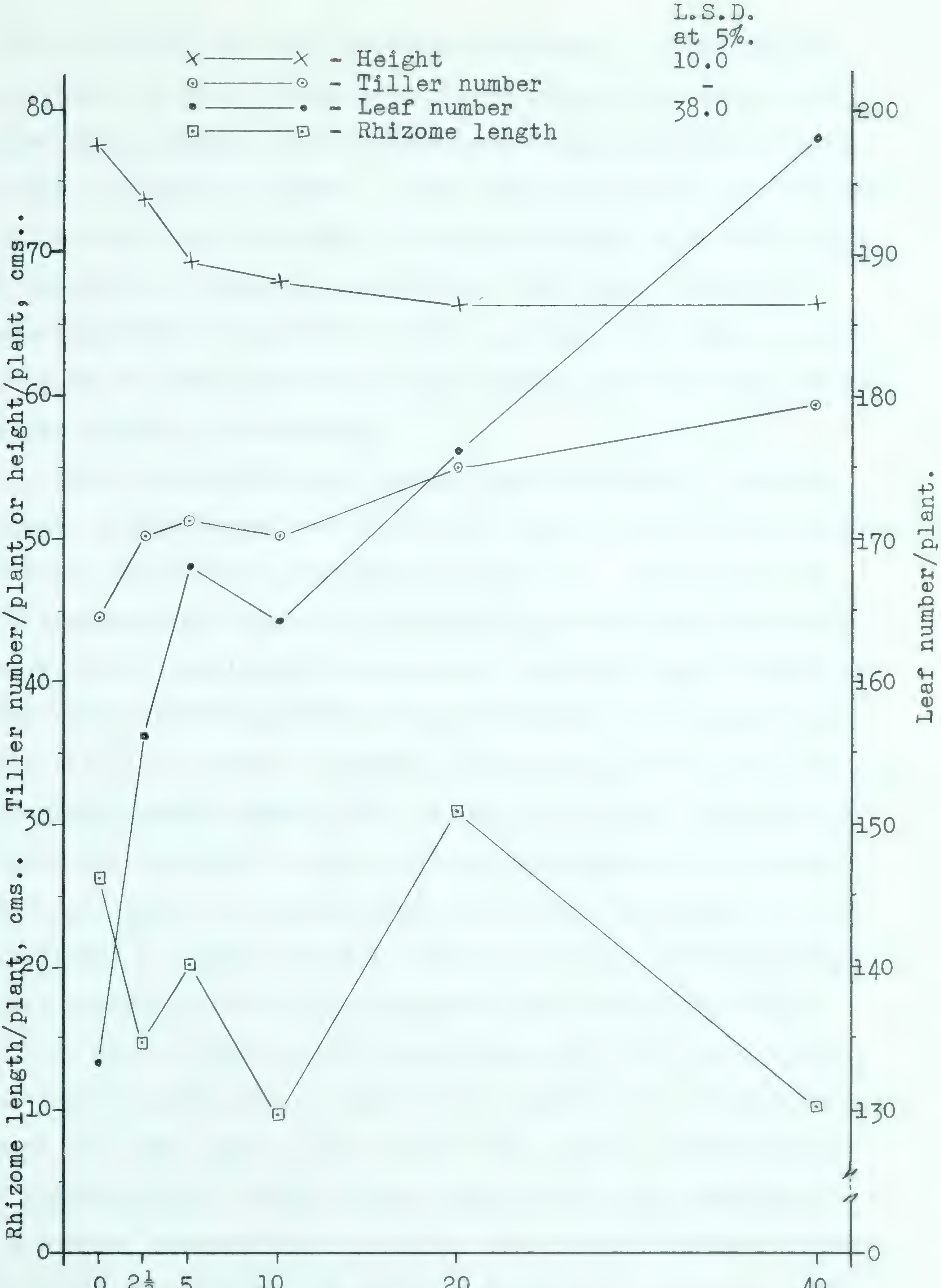


Fig. 13. Effect of CCC on height, tiller and leaf number and rhizome length of creeping red fescue.
(Data used - mean of 12 replicates; harvested 10 weeks after spraying).

similar to that used for Kentucky bluegrass. Spraying was done when the plants commenced tiller production, about four weeks after sowing, and the experiment was terminated after a further 10 weeks of growth. The rate of 40 lbs./a. of CCC was included so that the effect of this relatively high dose could be assessed on creeping red fescue. No signs of toxicity were observed at any level of CCC; creeping red fescue appeared to be the most tolerant to high dosages of CCC of the three forage species investigated.

Data obtained from the measurements of height, rhizome length, tiller number and leaf number at the end of the experiment are graphically presented in Fig. 13.. The effect of CCC treatment on height of creeping red fescue was relatively small, but a consistently increasing reduction was recorded as the rates of CCC application were increased. In comparison with the 25-30 percent reduction in height recorded for Kentucky bluegrass plants treated with 20 lbs./a. of CCC, creeping red fescue was reduced in height by only approximately 15 percent. This difference in response can probably be attributed to the difference in growth habit of the two grasses; Kentucky bluegrass normally produces an elongated stem during vegetative growth, which creeping red fescue does not, and thus measurements for height made on the latter species were, in the main, those for leaf length rather than stem length. Measurements of rhizome length showed extreme variability, no consistent trend being produced by increasing dose of CCC, although there were indications that CCC caused a reduction in length. This again may be attributable to the growth habit of the grass, as this species normally spreads by stolons rather than by rhizomes

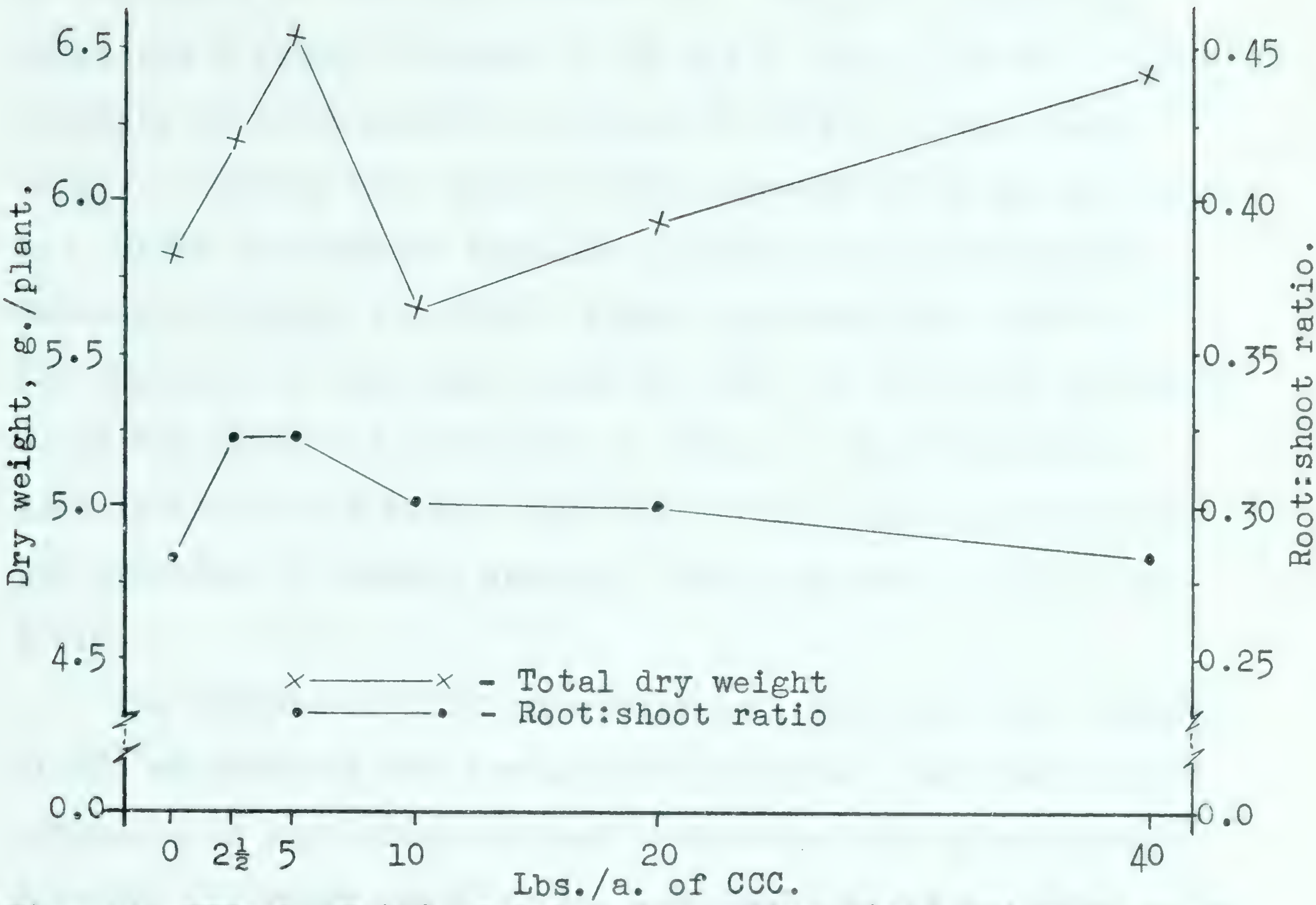


Fig. 14. Effect of CCC on total dry matter yield and root:shoot ratio of creeping red fescue.
(Data used - mean of 12 replicates; harvested 10 weeks after spraying).

and thus does not rely on the latter, which may or, as occurred in several instances, may not be produced. Tiller production was slightly, but not significantly, increased over that of the controls, and the closely related leaf number was considerably increased, the increase being significant at the 1 percent level. Weekly measurements of the above described morphological characteristics showed that trends for the development of the effects of CCC were similar to those for Kentucky bluegrass, with a maximum being recorded at about four to six weeks after spraying.

The total dry matter yield was not appreciably affected by treatment of the plants with CCC. Fig. 14. shows that there was a slight increase at $2\frac{1}{2}$ and 5 lbs./a. of CCC, followed by an unaccountable decrease at 10 lbs./a. and very slight increases over those of the controls at 20 and 40 lbs./a.; these differences were not statistically significant. Root:shoot ratios did show a slight increase over those of the controls at the lower rates of CCC, but at 20 and 40 lbs./a. of CCC there was little or no effect. No attempt was made to obtain dry weight data for the rhizomes, as they were non-existent in several samples, including those of the controls.

The results of this experiment indicated that the effects of CCC on creeping red fescue were not great, but that slight increases in tillering and leaf production with a concurrent decrease in height, similar to, but less pronounced than, those for Kentucky bluegrass, could be expected. It is possible that a greater effect of treatment with CCC would be obtained under winter conditions, as the above described experiment was carried out during the spring and early summer. The general small effects of CCC observed for this experiment also tend to confirm the insignificant effect on yield that was obtained for the latter Kinsella field experiment.

(5) Effect of CCC on the morphological development of brome grass.

Two experiments were conducted to investigate the effects of CCC on the morphological development of brome grass. The

first experiment, as previously mentioned, used plants grown from 'seed'. The results from this experiment proved to be extremely variable and therefore the second experiment was carried out using plants grown from rhizome sections obtained from a single clone, in an attempt to reduce the inherent genetic variation. The results of this experiment, which follow, showed that those obtained from the first trial, although variable, were closely similar and apparently typical of the effect of CCC on this grass. It should be pointed out that both experiments were conducted during the summer and early fall months.

The plants grew rapidly from the rhizome sections and started to develop tillers after about three weeks, at which time they were sprayed. Considerable scorching of the sprayed leaves occurred at 10, 20 and 40 lbs./a. of CCC and a few plants receiving the two higher levels died. This was contrary to expectations, as no toxicity had been observed for the plants grown from 'seed'. Removal of the dead plants revealed that although there had been a rapid development of the tops, only slight root production had occurred, and it is possible that this lack of root development contributed to the extreme effects observed.

Results of measurments made on plant height, rhizome length, tiller number and leaf number are presented graphically in Fig. 15.. It would appear from these results that CCC treatment had no effect on plant height. These data, however, were obtained 12 weeks after spraying, and Fig. 16., with data obtained every second week throughout the experiment, shows that the treatment of brome plants with CCC

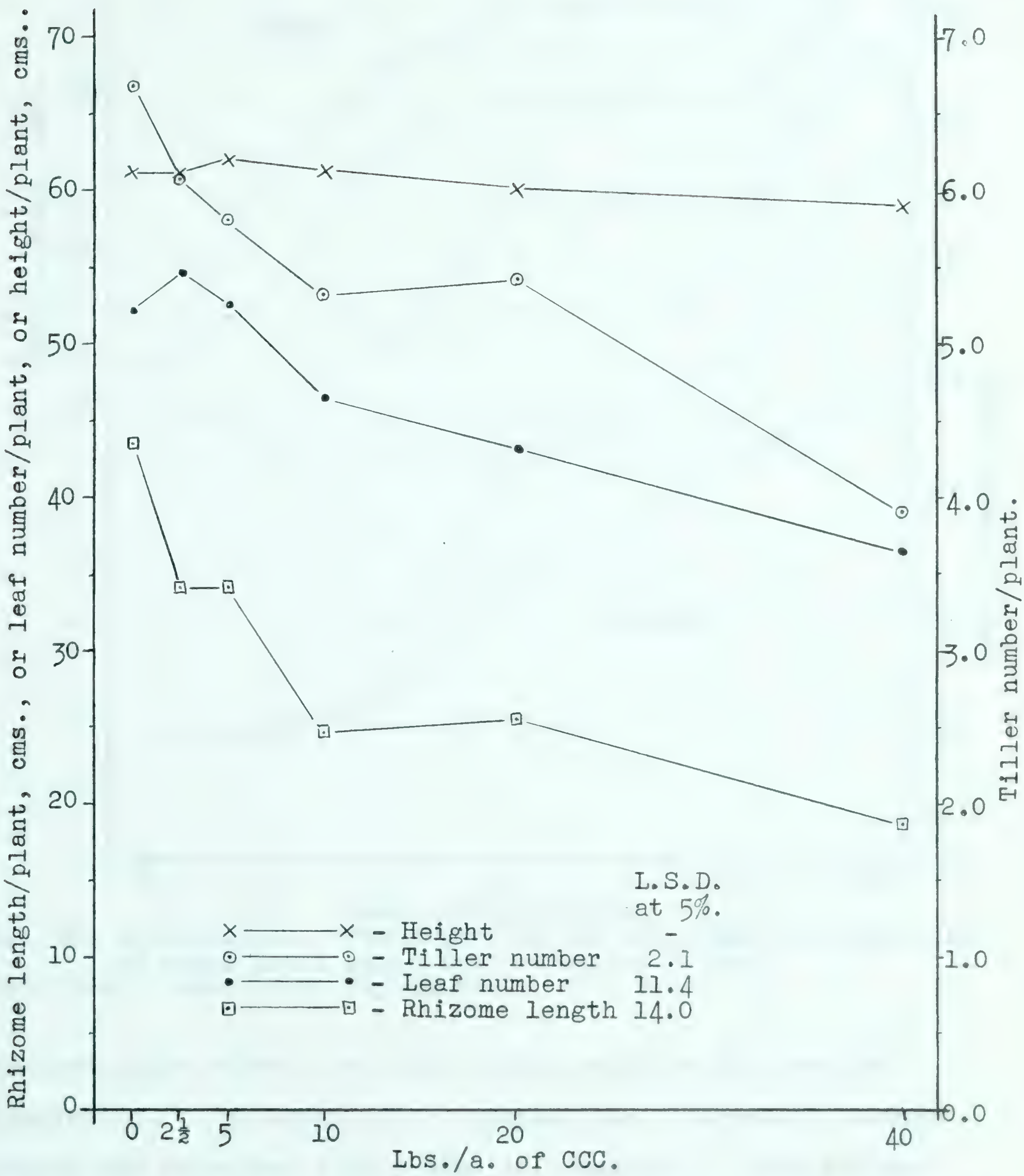


Fig. 15. Effect of CCC on height, tiller number, leaf number and rhizome length of brome grass grown from rhizome sections. (Data used - mean of 12 replicates; measured 12 weeks after spraying).

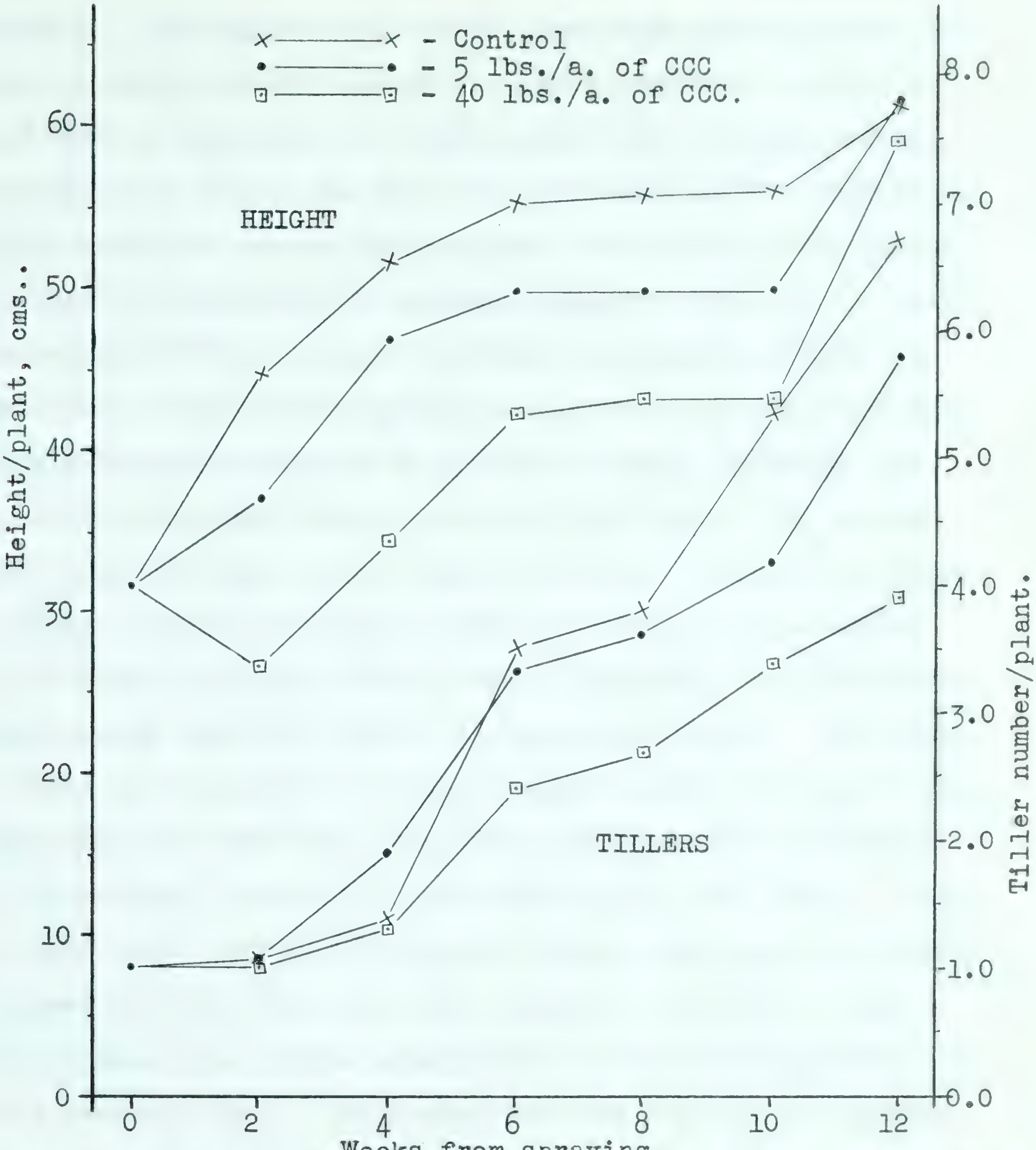


Fig. 16. Development of the effect of CCC on height and tillering of brome grass grown from rhizome sections. (Data used - mean of 12 replicates).

produced large effects on plant height prior to the terminal observation. Two weeks after spraying the differences between treated and untreated plants were at a maximum. This was due to the apparent decrease in height that had occurred at the higher rates of CCC application (because only living tissue was

measured; the higher rates of CCC producing severe scorch and death of many sprayed leaves). Plants from all treatments then grew at approximately equal rates until 10 weeks after spraying, but during the last two weeks all the CCC-treated plants grew faster than the controls, resulting in the apparent lack of effect of CCC treatment shown in Fig. 15.. Thus the effect of CCC on height of brome, in comparison with the other two species investigated, was greater and developed more quickly during the stages of growth following spraying, but appeared to be more easily nullified with time. It is possible that this was due to brome's inherent tendency for rapid elongation during the early stages of growth, thus enabling CCC to exert a greater effect than is possible on a relatively slow growing species, such as creeping red fescue. The apparent loss of the effect of CCC on height towards the end of the experiment may have been caused by the change from vegetative to reproductive growth that was occurring at that time. Fig. 15. also shows that CCC treatment reduced the length of rhizomes produced in comparison with the controls, the effect being considerable even at the lower doses of CCC and significant at the 1 percent level. Tillering was also found to be reduced by all levels of CCC, an effect opposite to that observed for the other two species investigated. The use of the term 'tillering', however, may not be fully justified with regard to the production of secondary growth in brome, as the majority of this is developed from the rhizomes. Each brome plant normally produces two basal tillers, and it can be seen from Fig. 16. that treatment of the plants with CCC at the lower rates had the effect of inducing rapid growth of these true

tillers, but that when these three shoots (two tillers plus the main stem) had been produced, at about six weeks after spraying, the plants relied on new shoots to be produced from the rhizomes. Thus, as the CCC appeared to suppress the development of the latter, it also lowered lateral shoot production. This explains why CCC treatment appeared to reduce tillering rather than increase it. Leaf number was increased slightly over that of the controls at the lower doses, but was decreased at the higher levels of CCC (Fig. 15.), probably because of the reduction in tillering being relatively large at these levels.

Table IX. Effect of CCC on leaf length, leaf width, leaf dry weight and stem diameter of brome grass grown from rhizome sections.

	Lbs./a. of CCC						L.S.D.
	0	$2\frac{1}{2}$	5	10	20	40	at 5%.
Length/leaf, mms.	23.6	22.9	24.8	24.2	22.8	23.2	-
Width/leaf, mms.	7.1	7.6	8.4	7.7	7.7	7.2	1.0
Dry weight/leaf, mgs.	19.8	20.4	22.6	20.5	17.9	16.8	1.1
Diameter/stem, mms.	1.9	2.1	2.1	2.3	2.2	2.1	-

Data used - mean of 12 replicates; harvested 8 weeks after spraying.

Leaves were removed from the plants in both experiments in order to obtain measurements of their chlorophyll content. The leaves used for this purpose were, wherever possible, the sixth fully expanded leaf from the apex of the main stem and the fourth fully expanded leaf from the largest lateral shoot; they were cut off at the junction of the leaf blade and the

sheath. Measurements were made of the leaf length, from the tip to the top of the leaf sheath, and width, at approximately five cms. from the junction of the leaf blade and the sheath. Dry weights were obtained after drying the leaves on a Virtis freeze-drier. The stem diameter, between the first and second visible main stem nodes, was also measured. The data obtained from the above described measurements are shown in Table IX.. Leaf length did not appear to be affected by treatment of the plants with CCC, but the width was increased somewhat over that of the controls; the greatest effect being at 5 lbs./a. of CCC. The data for the leaf dry weight showed that the lower rates of CCC produced slight, but significant, increases in comparison with those for leaves from untreated plants, and that the higher rates of CCC produced an equally small reduction in dry weight. Based on these figures, low rates of CCC appear to cause plants to produce slightly larger and heavier leaves, which is in accordance with previous reports (18,19,59); the higher rates of 20 and 40 lbs./a. of CCC tended to produce leaves that were reduced in size and weight. The measurements of stem diameter, although not possessing significant differences, showed a slight increase after treatment of the plants with CCC; this is in accordance with the increased dry weight per cm. previously recorded for Kentucky bluegrass rhizomes.

The data obtained from the dry matter yields are presented in Fig. 17., and show, as could be expected from the effects of CCC observed on the morphological characteristics, that the total dry matter yield was reduced by CCC treatment in comparison with that of the controls, except for a slight, non-significant, increase at the lowest rate of 2½ lbs./a. of CCC.

The root:shoot ratios also showed a tendency for decrease with increasing dose of CCC, although a smooth trend was not produced. This was in contrast with the results obtained for creeping red fescue and Kentucky bluegrass, on which CCC appeared to have little effect or to produce an increase; there does not appear to be a logical explanation for this apparent anomaly. The rhizome dry weight per cm. was increased over that of the controls by all levels of CCC, which was in agreement with the

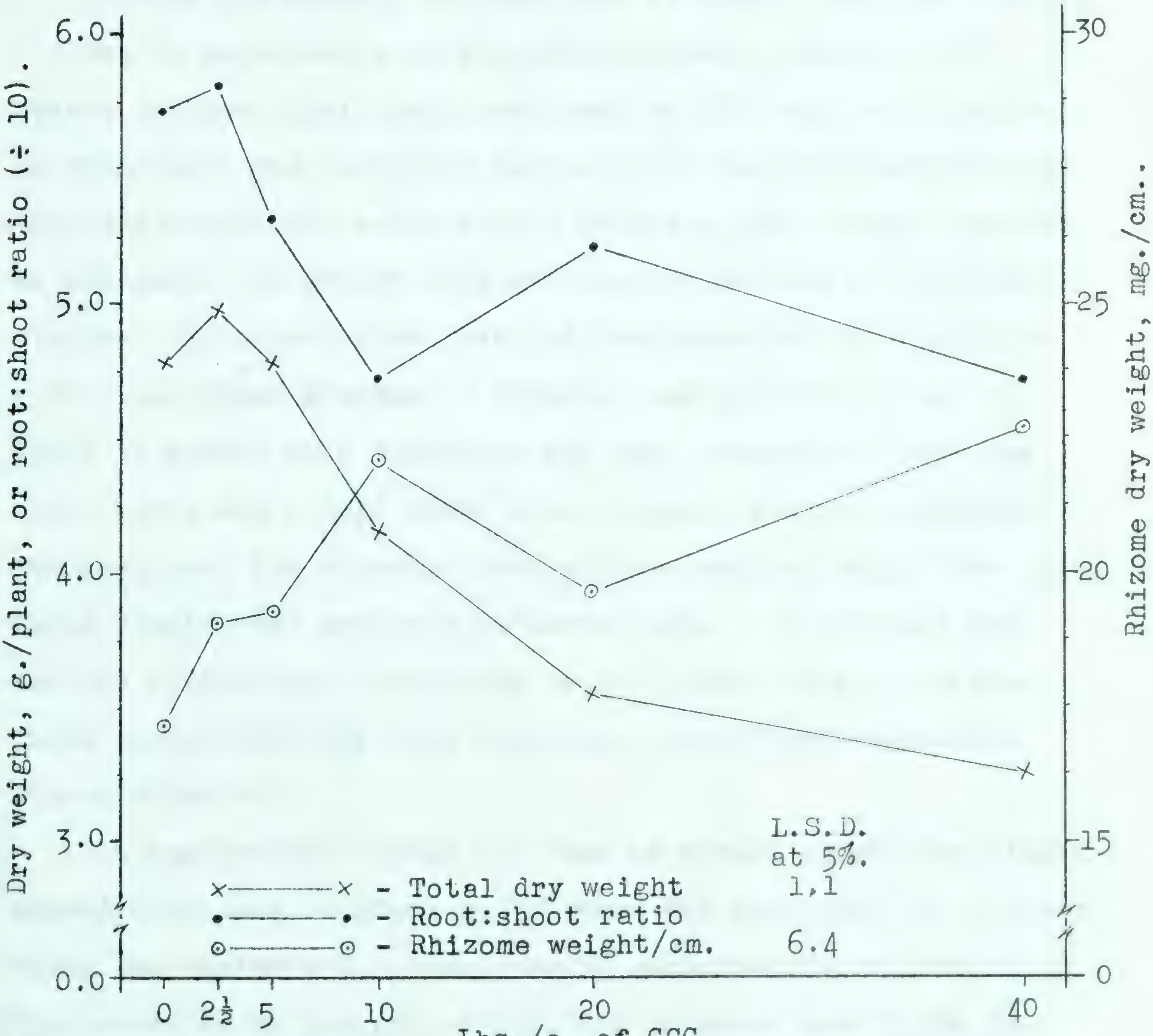


Fig. 17. Effect of CCC on total dry matter yield, rhizome dry weight per cm. and root:shoot ratio of brome grass grown from rhizome sections.
(Data used - mean of 12 replicates; harvested 12 weeks after spraying).

findings for Kentucky bluegrass rhizomes and for the results obtained for the measurements of brome stem diameter. The data concerning the effects of CCC on the dry matter yield of brome grass grown under greenhouse conditions thus confirmed those obtained under field conditions at Ellerslie.

(6) Effects of CCC on the morphological development of wheat, oats and barley.

During the growing of seedlings of wheat, oats and barley for use in experiments on the physiological effects of CCC, it became apparent that their responses to CCC were not identical. An experiment was therefore conducted in the greenhouse during the late winter and early spring on these three cereal species to determine the effect that CCC exerted on some of the morphological characteristics that had been measured for the three perennial forage grasses. Spraying was carried out at the stage in growth when tillering had just commenced; this was about three and a half weeks after sowing, and the experiment was continued for an additional eight weeks, at which time all three species had produced inflorescences. No attempt was made to obtain data pertaining to the latter as all the previous experiments had been concerned solely with vegetative characteristics.

It was obvious within one week of spraying that oat plants showed much less response to CCC than did the other two species. Wheat and barley both showed severe scorching and chlorosis of the leaves at 20 lbs./a. of CCC, and on wheat even 5 lbs./a. caused considerable scorch; in comparison with this, the oats appeared to suffer no adverse effects. The data obtained two

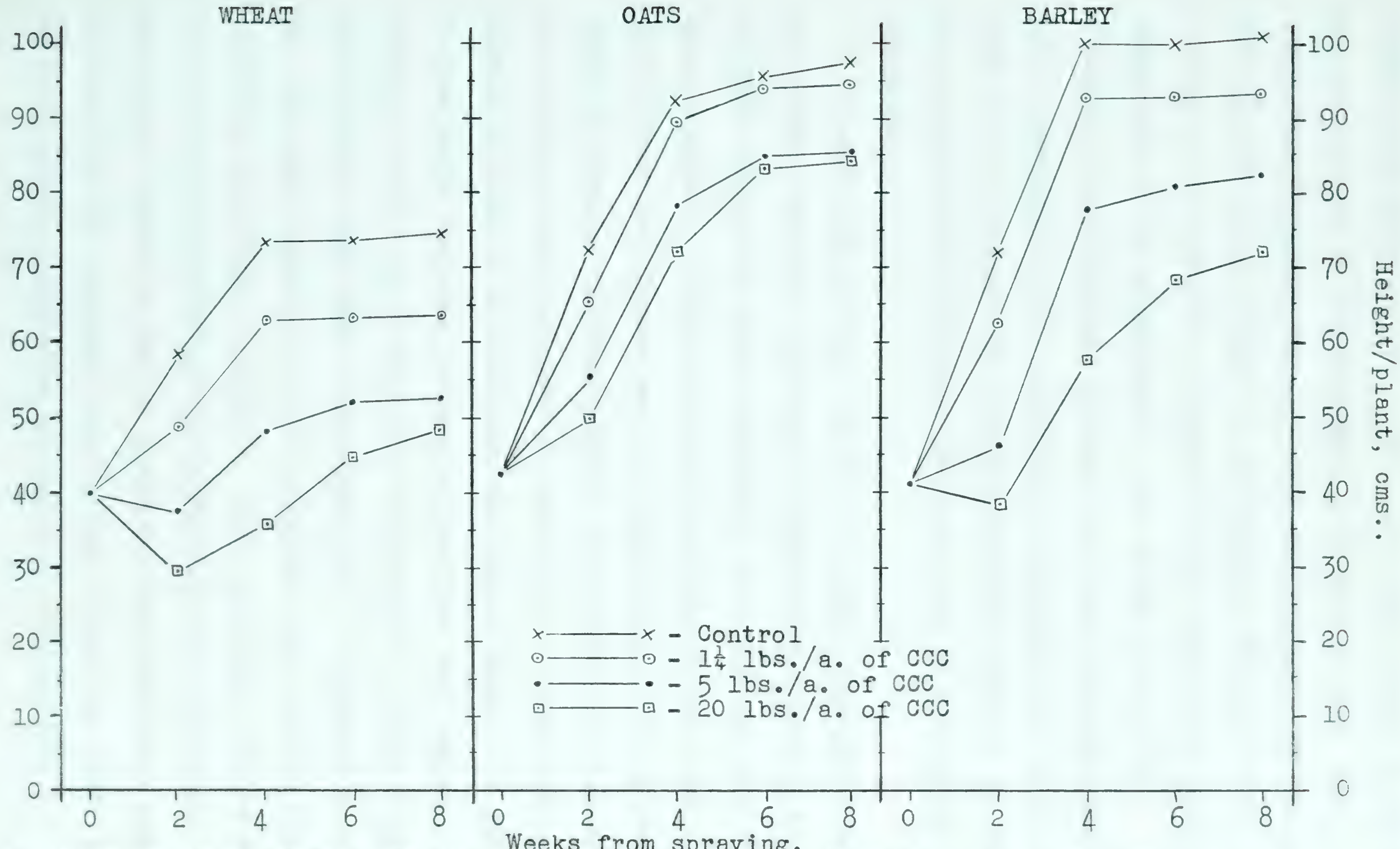


Fig. 18. Comparison of the effects of CCC on the height of wheat, oats and barley. (Data used - mean of 6 replicates.).

weeks after spraying (Fig. 18.), show the effect of CCC as it was manifested in plant height. No plants died, but in two replicates of wheat the main stem was killed by 20 lbs./a. of CCC, growth being resumed in the form of tillers. The magnitude of the effects on height did not change throughout the remainder of the experiment; also shown in Fig. 18.. A factorial type analysis on data for height obtained four weeks after spraying showed a significant interaction, at the 1 percent level, between the species and their response to CCC, but this significant difference had been lost by the end of the experiment.

Counts made on leaf and tiller number throughout the experiment revealed a pattern of steadily increasing effects of CCC, similar to those previously described for the forage grass species. Data obtained at the end of the experiment showed that leaf number was not affected by CCC treatment for wheat or barley, but was slightly increased over that of the controls for oats, as shown in Table X a.. The results presented in Table X b. show that the tiller number did not follow quite the same pattern as that for leaf number. Wheat treated with 1¼ or 5 lbs./a. of CCC showed a slight increase in tillering over that of the controls. Tillering of barley was decreased somewhat by CCC treatment and oats did not appear to have been affected. Thus, from the effects of CCC on the visible morphological characteristics of height, leaf and tiller number it can be concluded that oats were more tolerant to CCC treatment than either of the two other species, of which wheat appeared to be the most susceptible.

Table X. Effect of CCC on leaf and tiller number per plant, and on root:shoot ratios of wheat, oats and barley.

		a Leaf no./plant			b Tiller no./plant			c Root:shoot ratio		
		W	O	B	W	O	B	W	O	B
Lbs./a. of CCC.	0	11.2	18.3	11.5	3.8	9.3	5.0	0.10	0.10	0.10
	1¼	13.3	19.0	11.3	5.2	9.3	4.7	0.13	0.11	0.12
	5	11.0	20.3	11.3	4.0	9.3	4.7	0.12	0.12	0.11
	20	11.5	21.0	11.5	3.8	10.0	4.2	0.12	0.12	0.12

Data used - mean of 6 replicates; measured 8 weeks after spraying.
- W = wheat; O = oats; B = barley.

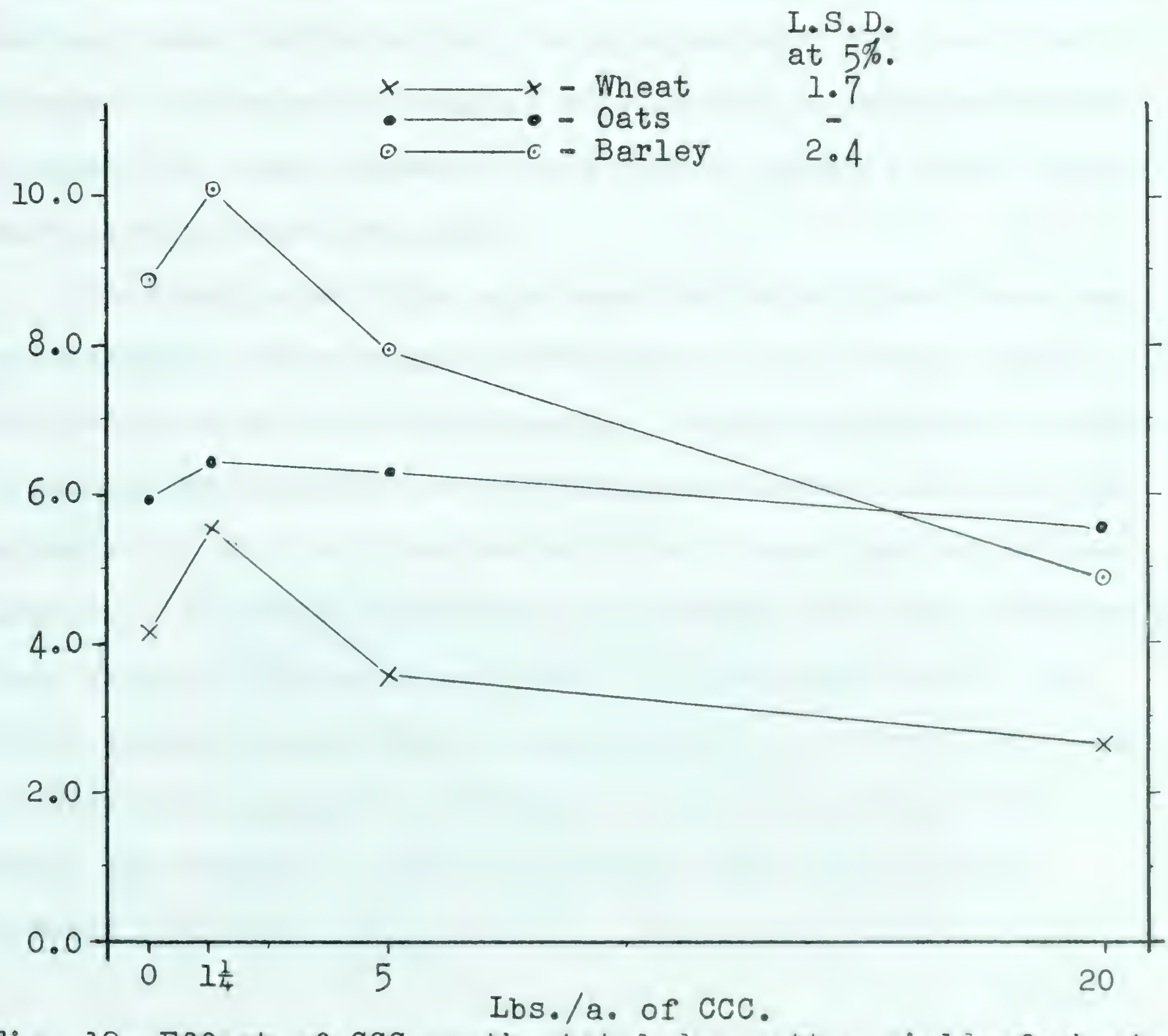


Fig. 19. Effect of CCC on the total dry matter yield of wheat, oats and barley.
(Data used - mean of 6 replicates; harvested 8 weeks after spraying)

The data obtained for the total dry matter yields of the three species are graphically presented in Fig. 19., and showed that CCC treatment had the least effect on oats; slight increases were recorded for 1¼ and 5 lbs./a. of CCC over that of the controls and an equally slight decrease at 20 lbs./a. Wheat and barley exhibited basically similar responses with regard to total dry matter production, the nature and degree of this response depending on the dose applied. An increase over the yield of the controls was obtained at 1¼ lbs./a. of CCC, a slight decrease at 5 lbs./a. and a considerable, statistically significant, reduction at 20 lbs./a.. Root:shoot ratios, shown in Table X c., were calculated for the three species; there were no major differences in response between them and all three showed a very slight increase after treatment of the plants with CCC.

The results of this experiment indicated that there are considerable differences in response to CCC between closely related species in the Gramineae. These differences did not appear to be dependent on differences in growth habit as had appeared to be the situation with the forage species investigated. It can be tentatively concluded from this experiment that slight differences may exist in the metabolism of the three cereal species which alter their response to CCC. It should also be pointed out that the results obtained for wheat are similar to those previously reported for this species (53).

(7) Effects of CCC on the early development of corn and wheat seedlings.

Measurements made on some of the morphological characteristics affected by the inclusion of CCC in the growing medium of seedlings prepared for studies of physiological differences attributable to the chemical, comprise this part of the thesis. Corn plants, Zea mays hybrid var. Wheatland, in preparation for determination of the effect of CCC on chlorophyll production were, as previously mentioned, grown in the dark with concentrations of 0, 100, 500, 1,000, 2,500, 5,000 and 10,000 ppm. of CCC included in the water. Fig. 20. shows the appearance of corn plants grown for 14 days in the above concentrations of CCC. It had been expected that 10,000 ppm. might prove to be toxic, but the plants in this concentration appeared to be completely healthy and showing none of the signs of etiolation that had developed in the control plants and those in the lower concentrations of CCC. In general, plants having CCC included in the water appeared to be more sturdy and nearer in appearance to that of light grown plants (except for lack

Fig. 20. Effect of CCC on 14-day old dark-grown corn seedlings.

of chlorophyll) than those grown in water only.

The height of the tops, from the first node to the tip of the longest leaf, was measured, revealing a steadily increasing effect for reduced height by a logarithmically increasing dose of CCC, as shown in Fig. 21.. Measurements made in all the other experiments involving corn seedlings showed equally consistent effects of CCC in this respect. It should be pointed

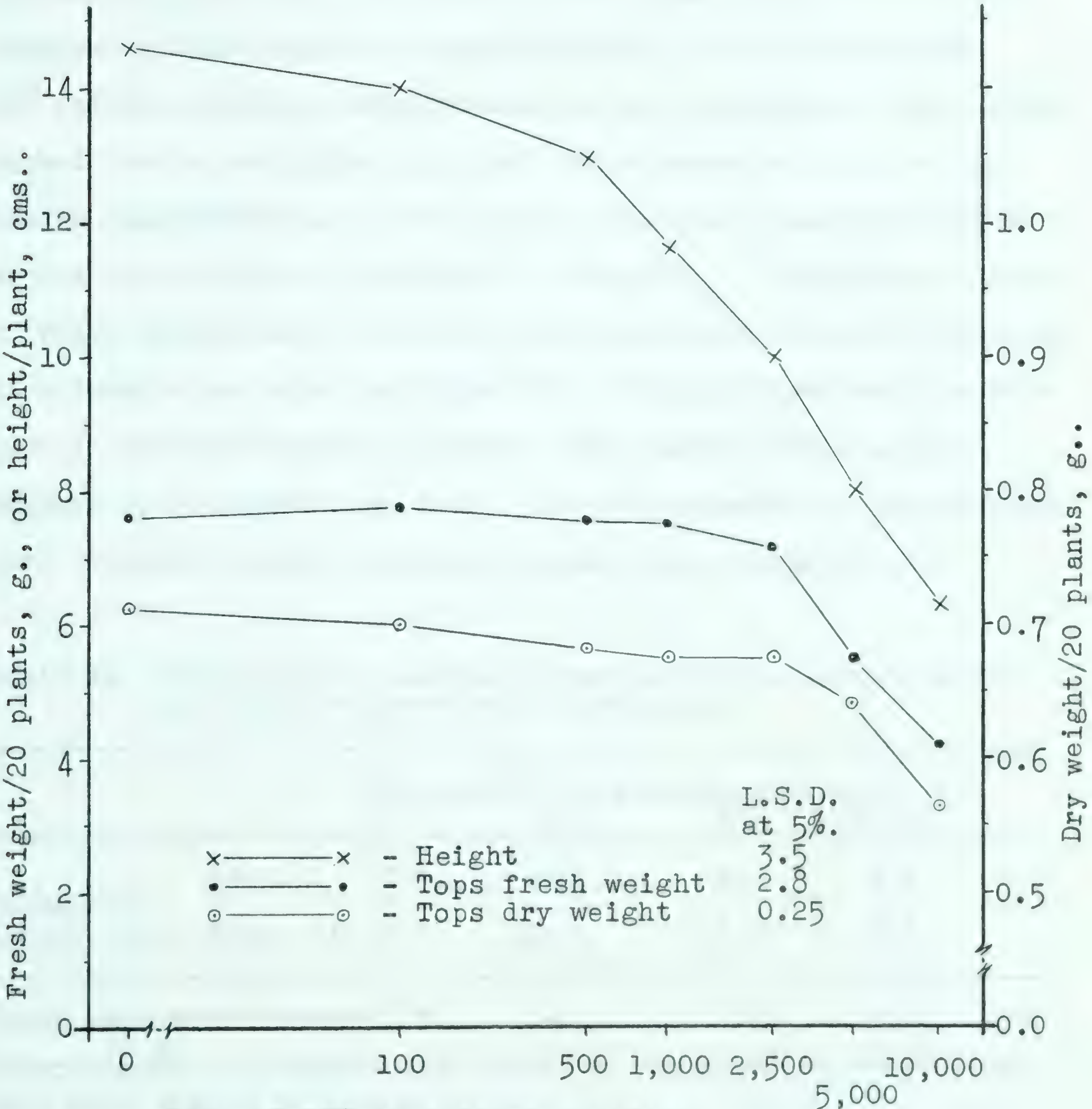


Fig. 21. Effect of CCC on height, fresh and dry weight of 14-day old dark-grown corn seedlings.
(Data used - mean of 4 replicates.).

out that concentrations of CCC as low as 10 ppm. produced slight, but measurable, effects on height; below this level no effects ould be detected.

The possibility that the observed changes in chlorophyll content, to be described in the next section of this thesis, were only the results of changes in plant volume was considered. Plant volume was measured as displacement of water in a narrow, graduated glass tube, in order to determine whether CCC caused changes in this aspect of the morphology of corn seedlings. The results of these measurements on two preliminary experiments showed that a reduction in plant volume occurred only at the higher concentrations of CCC, there being no consistent effect at the lower levels, as shown in Table XI.. Measurements made of fresh weight were found to correspond very closely with those of volume, also shown in Table XI., if millilitres were considered to be equivalent to grammes fresh weight (this approximation is relatively accurate, as the untreated corn seedlings were found to consist of 90-92 percent water when fresh).

Table XI. Effect of CCC on the volume and fresh weight of 14-day old dark-grown corn seedlings.

		Ppm. of CCC in culture solution. 0	50	500	5,000
Volume/20 plants, mls.	Expt. I	8.0	7.4	8.1	6.2
	Expt. II	10.0	10.5	10.6	8.6
Fresh weight/20 plants in g.		7.70	-	7.66	5.47

Data used - mean of 4 replicates.

Data obtained for the fresh weight showed that CCC did not affect it until concentrations of 2,500 ppm. or higher were used, when statistically significant (at the 1 percent level) reductions in comparison with the controls were produced, as shown in Fig. 21.. A general lack of effect of CCC treatment on the dry weights was recorded at the lower levels of CCC, but highly significant (at the 1 percent level) reductions occurred at 5,000 and 10,000 ppm. of CCC. As the changes that occurred in the dry matter yields of plants grown in 5,000 and 10,000 ppm. of CCC were relatively small, although significant, and since for the same plants there had been a relatively larger reduction in the corresponding fresh weight, they must have contained more dry matter per unit volume. These plants were found to consist of 85 and 87 percent water respectively when fresh, in comparison with the 92 percent of the untreated plants. This is of considerable interest and might have some bearing on the results obtained for increased tolerance of wheat seedlings (37,38) and soybeans (34) to extremes of pH and salt concentration. No further data, however, were obtained on this aspect, other than repeated experiments showing effects similar to those outlined above.

Thus these measurements of corn seedlings grown in CCC solutions showed that the apparently typical reduction in height occurred, although in this instance it could not be attributed to suppression of internode elongation. They also indicated that CCC did not alter the amount of food materials translocated from the old seed to the seedling and that a possibly significant reduction in water content of the living plants was effected at high concentrations of CCC.

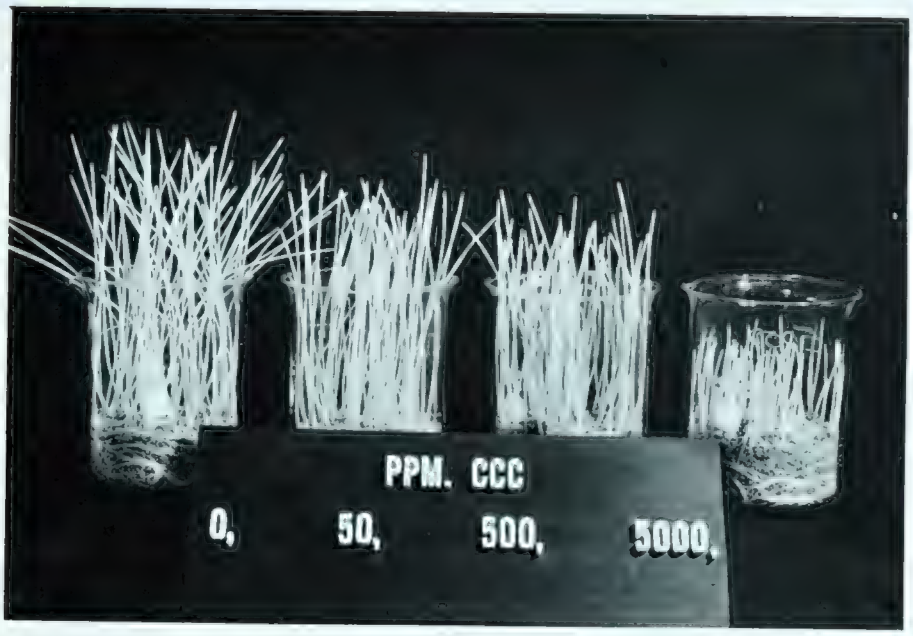


Fig. 22. Effect of CCC on 11-day old dark-grown wheat seedlings.

Wheat seedlings (Triticum vulgare var Thatcher) that were being grown for the studies on the effects of CCC on native auxin levels were also measured for changes in morphological characteristics. The range of CCC concentrations used was similar to that for corn, except that difficulty was experienced in growing the wheat at the higher concentrations of 5,000 and 10,000 ppm. of CCC. Fig. 22. shows the appearance of wheat seedlings grown for 11 days in the dark in concentrations of 0, 50, 500 and 5,000 ppm. of CCC. It is apparent that the effect on height was considerable, increasing CCC concentration producing plants that appeared as if grown in the light (except for lack of chlorophyll), being much sturdier and less etiolated than those grown in water only. Measurements of the distance from seed to tip of the longest leaf confirmed that CCC caused a reduction in plant height. This response was found to be approximately linear when plotted against a logarithmic increase in CCC concentration, as shown in Fig. 23.. The dotted projection from 2,500 to 5,000 ppm. of CCC represents

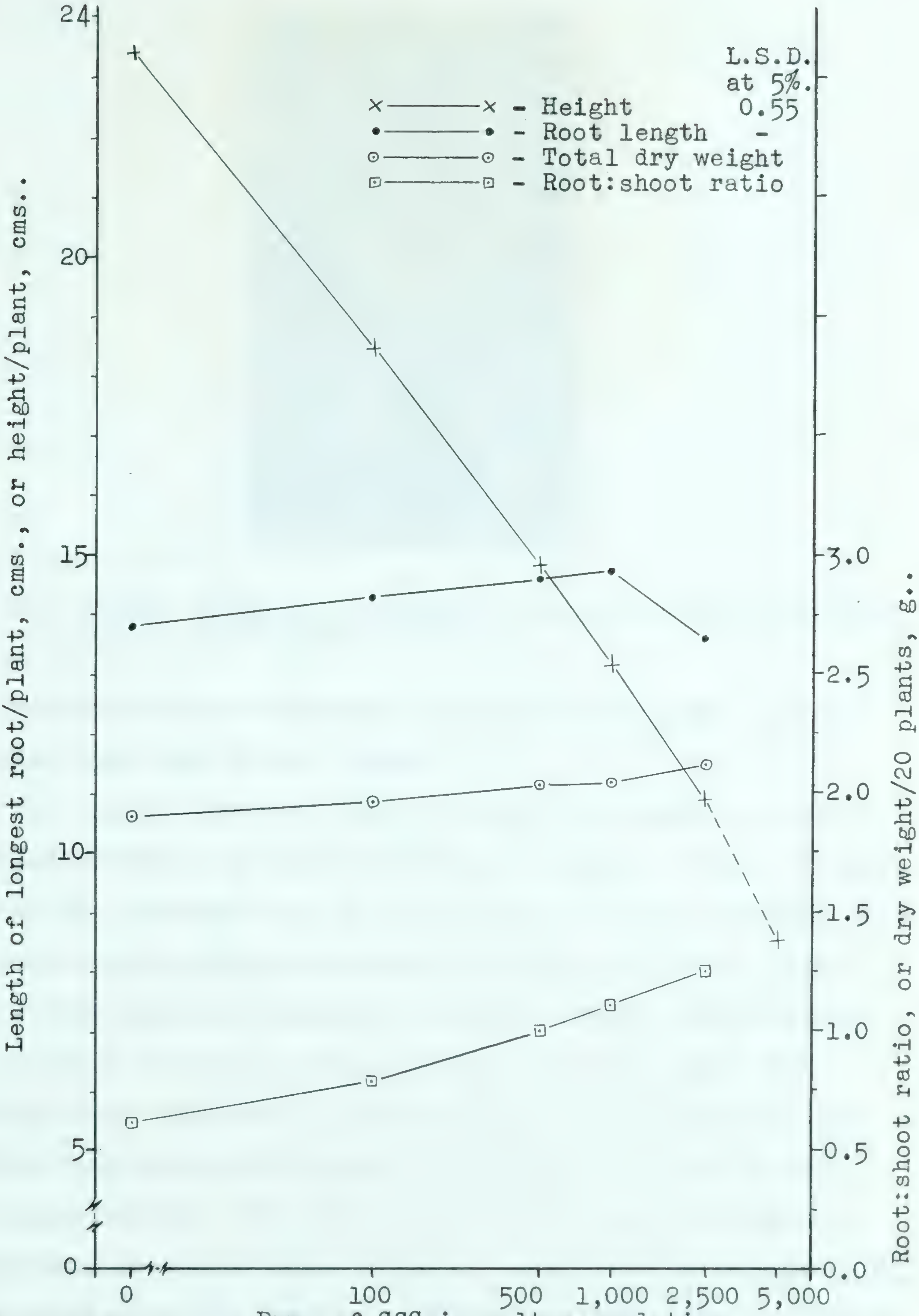


Fig. 23. Effect of CCC on height, root length, total dry matter yield and root:shoot ratio of 11-day old dark-grown wheat seedlings.
(Data used - mean of 4 replicates.).

Fig. 24. Effect of CCC on 11-day old dark-grown wheat seedlings, showing development of roots.

data obtained from a different experiment to the one used for all the other data in the figure.

The results obtained from measuring the length of roots produced proved to be of considerable interest. Fig. 23. shows that as the concentration of CCC in the water was increased, the length of roots produced increased slightly, except at 2,500 ppm. of CCC when the reduction in growth became sufficient to also cause a decrease in root growth. Fig. 24. shows the appearance of this almost complete lack of effect on root production, the plants photographed being representative of each CCC concentration. It will be immediately obvious from the graphically presented data in Fig. 23. that the root:shoot ratio, as calculated on the basis of root length/ tops length, increased steadily with increasing concentration of CCC. This proved to be the most clear-cut evidence that treatment of plants with

CCC increases the root:shoot ratio, apparently because it had little effect on root production whilst top growth was considerably suppressed.

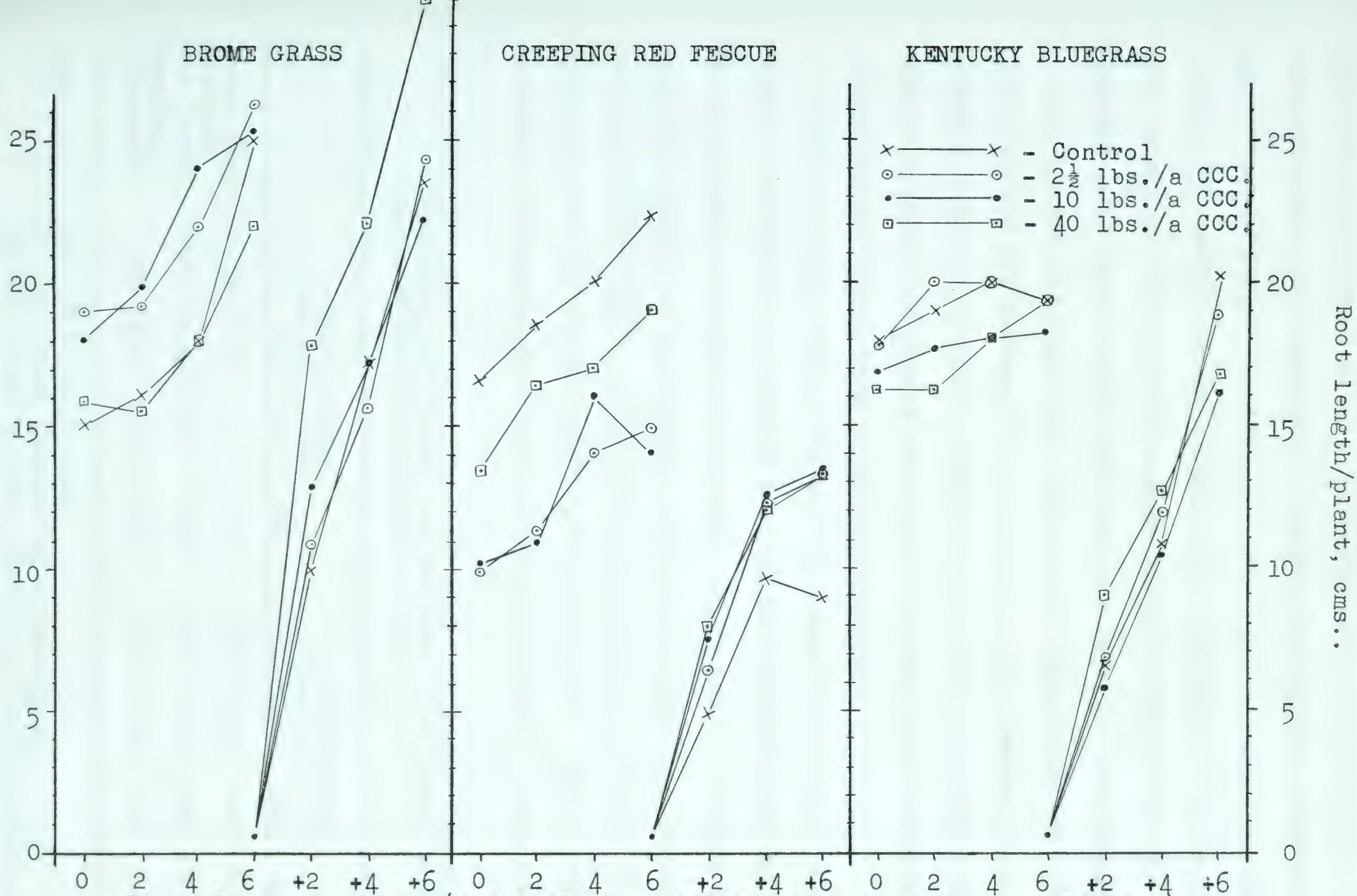
Data obtained for the dry matter content of the whole plants showed that CCC treatment resulted in a slightly, but statistically significantly, increased total dry matter content over that of the controls, as shown in Fig. 23.. Results of this nature, although not presented, were recorded for all the experiments conducted using wheat seedlings, and thus confirm the CCC-induced increase in total dry matter content just described. This would appear to indicate that CCC-treated plants had a generally lower metabolic, or probably more specifically, respiration rate than the controls, as all plants had been maintained in total darkness at a constant temperature throughout their growth. The only other possibility, as only vermiculite, water and CCC were used in the growth medium, was that CCC itself had been acting as a nutrient.

The measurements on wheat seedlings again revealed a suppression of height of the plants grown in a CCC solution, also an increased root:shoot ratio and what would appear to be a slightly lowered rate of respiration, although considerable further experimentation would be needed to verify the latter possibility. In the original work on wheat, Tolbert (53) mentioned that CCC was without effect in darkness or at light intensities of 10 ft-c. or lower. This would appear to be the opposite of the results presented above; Tolbert was, however, using slightly older plants grown in soil, and possibly this can explain the observed discrepancy.

(8) Effect of CCC on root production of brome grass, creeping red fescue and Kentucky bluegrass.

Although measurements had been made on root dry weight in all the experiments, this did not appear to be the most satisfactory method of studying the effect of CCC on root development, due to unmeasurable and probably variable losses incurred during sampling, except in the case of the wheat seedlings described in the last part of this treatise. In an attempt to measure root growth at regular intervals but without killing the plants, the specially constructed 'bottomless' pots, previously described, were used. This method was basically an adaptation of the 'ring culture' technique, the plants growing in the small pots with their main water absorbing roots in the medium below the small pots, in this case water. The roots growing through the mesh base of the pots and into the water below permitted weekly measurement of their length, and also clipping to obtain dry weight data without killing the plants. The three forage species, brome grass, creeping red fescue and Kentucky bluegrass, were used in this experiment. At the time of spraying the plants were well established, having been growing for approximately eight weeks. Fig. 25. shows that well developed roots were present at that time.

During the six weeks after spraying, measurements of root length showed no consistent effect of spraying with CCC, the root lengths in most treatments increasing at a rate which maintained differences already present at the time of spraying, as shown in Fig. 25.. After the first clipping the plants which had been treated with CCC produced more rapid root growth, this being a statistically significant increase for brome grass



Weeks from spraying. ('+' indicates additional weeks after 1st clipping).

Fig. 25. Effect of CCC on the development of roots of brome grass, creeping red fescue and Kentucky bluegrass, using special 'bottomless' pots.
(Data used - mean of 6 replicates).

and creeping red fescue, but too small and not so clearly defined for Kentucky bluegrass. These differences, although no longer statistically significant, were still evident, as shown in Fig. 25., at the final clipping six weeks after the initial one, except for an apparent suppression of root growth for Kentucky blue grass during the last two weeks. The decrease in root length observed for creeping red fescue treated with 10 lbs./a. of CCC between the fourth and sixth weeks after spraying, and the controls between the fourth and sixth weeks after the initial clipping were due to fungal, or bacterial, attack, which killed part of the root system.

The dry weight data, however, as shown by the root:shoot ratios in Table XII., did not confirm the apparent effects of CCC application recorded using root length data. Unlike the variable effects on root length recorded at the first clipping, the root:shoot ratios all showed an increase over those of the controls after treatment of the plants with CCC, except for creeping red fescue receiving 10 lbs./a. of CCC. The root:

Table XII. Effect of CCC on the root:shoot ratios of brome grass, creeping red fescue and Kentucky blue grass, grown using special 'bottomless' pots.

	1st clipping Lbs./a. of CCC.				2nd clipping Lbs./a. of CCC.			
	0	$2\frac{1}{2}$	10	40	0	$2\frac{1}{2}$	10	40
Brome grass.	0.16	0.18	0.17	0.20	0.07	0.06	0.07	0.14
Creeping red fescue.	0.10	0.11	0.07	0.10	0.03	0.06	0.06	0.06
Kentucky bluegrass.	0.11	0.12	0.12	0.13	0.06	0.06	0.05	0.05

Data used - mean of 6 replicates.
1st clipping÷ 6 weeks after spraying.
2nd clipping÷ 6 weeks after the first clipping.

shoot ratios obtained from the second clipping six weeks after the first one, did not show any consistent differences that could be attributed to treatment with CCC. Thus, although the root length produced after clipping was greater for plants receiving CCC than for those which did not, dry matter yield of the roots was not affected to such a great extent as the tops. These results, nevertheless, do confirm the general statement that CCC tends to increase the root:shoot ratio of treated plants in comparison with those of untreated plants. Although there were no difficulties in obtaining weights for the dry matter content of the roots using the above method, the procedure was not completely satisfactory due to the previously mentioned difficulty experienced in maintaining all the roots in a healthy condition.

Table XIII. Effect of CCC on tops dry matter yield of brome grass, creeping red fescue and Kentucky bluegrass used for root development studies and grown in special 'bottomless' pots.

	1st clipping Lbs./a. of CCC.				2nd clipping Lbs./a. of CCC.			
	0	$2\frac{1}{2}$	10	40	0	$2\frac{1}{2}$	10	40
Brome grass.	2.70	3.59	3.25	2.12	3.39	3.50	3.61	3.86
Creeping red fescue.	3.01	2.63	2.50	2.89	2.78	3.06	2.86	3.00
Kentucky bluegrass.	2.35	2.33	2.26	2.36	2.52	2.48	2.89	2.87

Data used - dry weight/pot, g., mean of 6 replicates.
1st clipping 6 weeks after spraying.
2nd clipping 6 weeks after first clipping.

The dry matter yields of the tops obtained from this experiment proved to be of considerable interest. The yields for the first clipping showed little effect of CCC, or slight

reductions, in comparison with those of the controls, for both Kentucky bluegrass and creeping red fescue, as shown in Table XIII.. This was in accordance with the previously described results for the study of the effects of CCC and clipping on these species. Brome grass, however, showed an increased dry matter yield for plants receiving $2\frac{1}{2}$ and 10 lbs./a. of CCC in comparison with that of the untreated plants, with a decrease at 40 lbs./a.. The increase was somewhat larger than might have been expected from the previously described effects of CCC treatment on the yield of brome, but the decrease at 40 lbs./a. of CCC was in accordance with these earlier findings. At the second clipping, as shown in the corresponding part of Table XIII., there was a general trend for increased dry matter yield over that of the controls for the plants that had originally been treated with CCC, although these differences were not statistically significant. These results therefore tended to confirm those obtained for the initial clipping experiment, to the effect that CCC would appear to increase the ability of treated plants to recover from clipping.

(9) Interaction of the effects of CCC and light intensity on Kentucky bluegrass.

Frequent mention has been made, relative to some of the previously described results, to the apparent decrease in the effect of CCC in experiments conducted during the summer in comparison with those during the winter, and similar effects have been reported by other workers (21,53). Both the latter reports suggested that the apparent loss of the effect of CCC was probably related to light intensity, in that treated plants

grown under low light intensities appeared similar to untreated ones grown under higher intensities. Two experiments were therefore conducted in a growth chamber, using four different light intensities (3,000, 2,000, 1,500 and 800 ft-c.) produced by filters of differing thickness constructed of alternating layers of glass and cheese-cloth, in an attempt to obtain data pertaining to this apparent interaction. Plants of Kentucky bluegrass were either left untreated or were sprayed with 10 lbs./a. of CCC, as this level had previously appeared to produce approximately optimal effects. Spraying was carried out at the onset of tillering, and the experiments were continued for 10 weeks. The temperature in the growth chamber was maintained at 20°C. and a day-length of 18 hours used.

The results from the first experiment have not been included in the thesis because they are subject to doubt due to a mechanical failure in the growth chamber one day after spraying. They did, however, show the same trends as those of the second experiment. The results of the second experiment, expressed as the percentage difference between the treated and the untreated plants grown at the four different light intensities, indicated that no consistent interaction between CCC and light intensity had occurred, as shown in Table XIV.. It should be pointed out that decreased light intensity, as could be expected, caused increased elongation of the stem, and decreases in all the other morphological characteristics measured. Height showed the expected reduction after treatment of the plants with CCC, but the magnitude of the effect did not appear to be influenced by light intensity. The results of the previous experiments had indicated that effects of CCC on this

Table XIV. Interaction of the effect of CCC and light intensity on Kentucky bluegrass; data obtained from plants receiving 10 lbs./a. of CCC being expressed as a percentage of those from untreated plants.

	Light intensity, ft-c. 3,000	2,000	1,500	800
Height	81	46	83	89
Rhizome length	58	65	67	59
Leaf number	192	137	122	121
Tiller number	110	120	126	108
Total dry weight	86	95	108	91

Data used - mean of 10 replicates; harvested 10 weeks after spraying.

characteristic were not altered to any great extent by winter or summer conditions, and thus the above result on plant height was not unexpected. Rhizome length was also reduced for CCC treated plants in comparison with the controls, and did appear to show a slight interaction with light intensity, as there was a tendency for an increased effect of CCC treatment at the intermediate light intensities, but not at the lowest. Leaf number was increased by CCC treatment, but tended to show a negative interaction with decreasing light intensity. Tiller production showed no consistent effect of treatment of the plants with CCC, there being a slight positive interaction with decreasing light intensity, except at 800 ft-c.. The total dry matter yield also showed a possible slight, non-significant, trend for a positive interaction of the effect of CCC treatment and light intensity. Results obtained from the first experiment, as mentioned previously, tended to confirm

those presented above for the second experiment. Thus, although there were possibly greater effects of CCC treatment at the two intermediate than at the highest light intensity on rhizome production, tillering and total dry matter yield, no definite conclusions can be drawn from these experiments. The apparent reversal of the trend for increased effect of CCC treatment with decreasing light intensity by the results obtained for plants grown at 800 ft-c. may be due to the fact that this intensity was too low, being insufficient to allow photosynthsis and other light sensitive processes to proceed sufficiently rapidly to permit the differences between treated and untreated plants to attain a maximum.

The possibility that duration of light may be more important than light intensity in determining the magnitude of the effect of CCC, and the lack of a consistent positive interaction between light intensity and CCC obtained in the above described experiment, prompt the suggestion that differences in duration of light may be the factor responsible for the observed differences in the effect of CCC between winter and summer light conditions. Although previous authors have found that CCC alters the response of plants to photoperiod (19, 48,63,64), light intensity should not be eliminated as a factor in determining the magnitude of the effect of CCC on plants, and before any definite conclusions could be drawn as to whether intensity or duration are more important, experiments with more accurately controlled light intensities should be performed. It is possible that the filters used in the above experiment affected the quality of the light and thus the response of the plants to it, and the use of filters con-

structured from polarizing material might give more useful results.

(10) Effect of CCC on the drought resistance of creeping red fescue.

Halevy and Kessler (17) found that CCC increased the drought resistance of beans, and the possibility that a similar effect might be exerted on grasses has been suggested in order to possibly explain some of the results obtained for the field experiments at the University of Alberta ranch at Kinsella. One experiment was therefore conducted in the greenhouse using methods similar to those of Halevy and Kessler, but with creeping red fescue. One major difference in technique was the use of spraying to apply the CCC for the present experiment, rather than the soil drench used by Halevy and Kessler.

An attempt was made to score the degree of wilting of the plants at various stages of the experiment. This proved impossible due to the difficulty of observing wilting for a grass such as creeping red fescue. The results for the dry matter

Table XV. Effect of CCC on the dry matter yield of creeping red fescue grown under drought conditions, and on the final soil moisture content.

	Lbs./a. of CCC.			
	0	$1\frac{1}{4}$	5	20
Dry weight of tops/pot, g.	10.35	10.45	10.57	9.12
Water remaining in soil, %.	5.0	4.7	5.1	5.0

Data used - mean of 6 replicates; harvested 6 weeks after spraying = 4 weeks after commencement of drought.

yields of the tops at the end of the experiment showed no significant effect of CCC or interaction with the drought regime, as can be seen in Table XV.. Thus, although this result confirms the previous findings that CCC causes only slight changes in the dry matter yield of creeping red fescue, there would not appear to be any effect on its drought resistance. Halevy and Kessler reported that the soil in which CCC treated plants had been grown contained less moisture at wilting point than that in which untreated plants had been grown. Measurements of percentage soil moisture at the end of the above experiment showed no differences between soil from pots containing treated or untreated plants, as shown in Table XV.. Although these limited results indicated that CCC does not affect the drought resistance of creeping red fescue and do not agree with those published by Halevy and Kessler, it must be remembered that there was a basic difference in the methods of application, which could account for the lack of effect observed in the above experiment.

EXPERIMENTAL III LABORATORY EXPERIMENTS

Materials and methods

(1) Chlorophyll determinations

Measurements of chlorophyll content were made on leaves obtained from CCC-treated and untreated brome grass plants. As described previously, this involved removing the sixth fully expanded leaf from the main stem and the fourth fully expanded leaf from the largest secondary shoot or tiller of each plant, these being cut off at the junction of the leaf blade and the leaf sheath. This was done five weeks after spraying in the first experiment and eight weeks after spraying in the second. Each sample of two leaves was cut into sections and stored in a stoppered 6x1 cm. glass vial at 2°C. until used.

The method employed to determine the chlorophyll content was basically that of Maclachlan and Zalik (33), and is outlined below. The fresh material obtained from the seed-grown brome experiment was weighed, cut into small pieces and placed in a mortar with a small quantity of fine, washed sand and $CaCO_3$, and ground. Three aliquots of approximately 10 mls. of pure acetone were used to extract the pigment from the ground sample. The extract was filtered, the brei washed with a further aliquot of acetone, and the total volume measured. If the extract could not be assayed immediately it was stored in a deepfreeze at approximately -25°C.; samples were allowed to return to room temperature after such storage before being assayed. A Beckman DK 1 recording

spectrophotometer was used to determine the absorption spectrum of three millilitres of each sample, or a known dilution of it. A 1 cm. path quartz cell was used and the absorbance was measured over the light wave-length range of 600 to 700 millimicrons (mμ). The calculations used were those derived by Maclachlan and Zalik and were:

for all green material:

$$Ca = \frac{(12.3xD_{663} - 0.86xD_{645})V}{d \times 1,000 \times W}$$

$$Cb = \frac{(19.3xD_{645} - 3.6xD_{663})V}{d \times 1,000 \times W}$$

and for the protochlorophyll determinations (described later)

$$Cp = \frac{(-0.144xD_{663} - 0.171xD_{645} + 1.006xD_{623})V}{0.035 \times d \times 1,000 \times W}$$

where C = concentration in mg./g. (fresh or dry weight); a = chlorophyll a; b = chlorophyll b; p = protochlorophyll; D = optical density at the wave-length indicated; V = total volume of extract, in mls. (including any allowance for dilution); d = lenght of light path, in cms. and W = weight (fresh or dry) of material, in g..

In the second brome experiment, in an attempt to remove variation that could arise from differences in water content of the leaves, they were freeze-dried on a Virtis freeze-drier prior to being ground and extracted. Ninety percent acetone was used for the extraction of the freeze-dried sample rather than 100 percent, as no water was present in the tissue. This slight modification in the technique was used throughout the remainder of the work.

In order to obtain further information concerning the effects of CCC on plant chlorophyll production, corn seedlings, grown as previously described, were exposed to one 24-hour cycle of light after being grown in the dark for two weeks, at which time the somewhat reduced third leaf was begining to expand. For the initial experiments, the light cycle was that of normal daylight in Alberta during March and April. This gave satisfactory results, but in order to provide more rigidly controlled conditions for the later experiments, the light cycle was supplied using a small growth chamber with a light intensity of 1,800 ft-c., from Sylvania 'cool white' type fluorescent tubes, and set for a day length of 14 hours. The plants were placed in the light at the commencement of a cycle and therefore remained in the dark for 10 hours after exposure to the light before sampling was carried out. Samples consisting of 20 plants each, cut at the first node, as chlorophyll did not develop below this point, were used throughout this work. After freeze-drying, the technique was the same as that previously outlined.

The methods described above were used to determine the chlorophyll production of corn seedlings grown to obtain data pertaining to the following: I). Effect of concentration of CCC. II). Effect of CCC on protochlorophyll production. III). Interaction between the effect of CCC and added ammonium chloride. IV). Interaction between the effect of CCC and the intensity of light used during the exposure cycle, and V). Interaction between the effect of CCC and the rate of loss of chlorophyll in the dark.

The experiment to determine the protochlorophyll content necessitated modifications of the technique for sample preparation; these are outlined below. After two weeks of growth in the dark with no exposure to light at any time, the samples were cut and prepared as usual, except that all the operations were carried out under a green safe-light. The latter precaution is necessary to eliminate the conversion of protochlorophyll to chlorophyll, which would occur rapidly under normal daylight laboratory conditions. The green safe-light was constructed from a single green Sylvania fluorescent tube mounted in a wooden housing, and filtered by three layers of no. 2092 green plexiglass and one layer of yellow lucite (plastics obtained from Layfield Plastics Ltd. of Edmonton). This safe-light had a peak transmission at 530 mμ. dropping to zero by 508 and 560 mμ.. The samples were transferred to the freeze-drier and dried, without exposure to light, and were ground and extracted under the green safe-light. The resulting acetone extracts were handled in the light as described for the other experiments. The low level of protochlorophyll in the samples necessitated the use of a 10 cm. path cell in the spectrophotometer, holding approximately 30 mls. of the extract.

(2) <u>Auxin determinations</u>

Initial studies of native auxin levels in CCC-treated and untreated seedlings were made on corn, which had been grown in a manner similar to that used for plants prepared for chlorophyll studies. Corn was subsequently found to be

unsuitable for these auxin studies and attempts were made to do similar studies using oats. As mentioned previously, however, oats were not affected by treatment with CCC to such an extent as were some of the other species. Accordingly wheat was finally chosen for the experiments to be described in this part of the thesis, and was grown using the methods outlined earlier. Sampling consisted of washing all the vermiculite from the roots and freeze-drying a known number of plants, either 50 or 100.

A Beckman DK 1 recording spectrophotometer was used to determine the auxin content of the samples, a slight modification of the technique for the estimation of IAA developed by Fletcher and Zalik (9) being employed; this modified technique is outlined below.

Each dried sample was weighed, cut into small pieces and placed in approximately 30 mls. of ice cold methanol in a special small volume attachment on a Waring Blendor. The homogenizing was carried out for five minutes at a Variac setting of 100 (max. setting 125), care being exercised at the start to avoid loss of sample due to splashing. The ground sample was then washed into a beaker and placed, in the dark, in an ice temperature water bath. After four hours it was filtered, the brei washed thoroughly and the final volume of the extract measured. All the above manipulations were carried out in a low temperature room maintained at approximately 3^{o}C.. The extracts were stored in a refrigerator at approximately 0^{o}C. until used.

Prior to any type of assay, the auxins in the sample were separated by means of paper chromatography. An aliquot rep-

resenting either 0.20 or 0.25 g. of the dried sample was pipetted into a 50 ml. flash evaporator flask and reduced to approximately 0.4 to 0.5 ml. on a flash evaporator. This small sample was carefully decanted into a 6x1 cm. glass vial, which was stoppered and allowed to stand in the refrigerator for approximately three hours. Using a Harrison microliter (µl) syringe the whole sample was applied across the start line of a 7.6 cms. wide strip of Whatman No. 1 chromatographic paper; the speed of drying the sample onto the paper was increased by the use of a hair dryer controlled from a foot operated switch. The reason for leaving each evaporated sample in the refigerator for approximately three hours was to allow the bulk of the sugary materials to be precipitated out. It was found that by doing this, clogging of the syringe by these materials, which occurred frequently if they had not been precipitated out, was almost completely eliminated. The chromatograms were then developed in a glass tank, at room temperature and in the dark, using the descending technique. The solvents used to develop the chromatogram varied with the object of the experiment. Identification of the positions of the auxins on the chromatogram was made on a 6.5 mm. strip cut from one side (equals 1/12 of the width of the chromatogram), the reagent para-dimethylaminocinnamaldehyde (DMAC), described by Fletcher and Zalik (9), being sprayed onto this strip with a glass atomizer. Three other spray reagents were used to confirm the identity of the auxins detected by DMAC. These were the Salkowski reagent (ferric chloride and perchloric acid), Ehrlich's reagent (para-dimethylaminobenzaldehyde, DMAB) and nitrous acid, all prepared according to the procedures describ-

ed by Leopold (29). Chromatograms that could not be used immediately were stored under vacuum in a deep-freeze; chromatographed auxins have previously been reported to store well under these conditions (27).

For the spectrophotometric determinations, a 2.5 cm. piece of the appropriate section of the chromatogram was removed, cut into small pieces and placed in a 30 ml. beaker. It was found that the accuracy of obtaining the desired area from the chromatogram was increased by the use of an ultraviolet lamp, which makes visible bands of fluorescence appear on the chromatogram, permitting allowances to be made during cutting for uneven solvent flow rates and hence non-straight bands of material. The sample was eluted from the chromatogram paper with 4 mls. of pure methanol by shaking for 10 minutes on a mechanical shaker. Three mls. of the eluted sample were transferred to a 1 cm. path quartz cell and its absorbance measured on the spectrophotometer in the ultraviolet region of the spectrum, between 300 and 240 mμ.. The readings thus obtained were then compared with those of a known concentration series of synthetic IAA (or L-tryptophan), and the quantity present determined.

In order to obtain data on the effects of CCC on the growth promoting auxins present in wheat seedlings, a bioassay of the chromatograms was employed. The bioassay technique used was that developed by Nitsch and Nitsch (41), using first internode sections cut from three-day old oat seedlings, var Brighton. The seed was washed for two hours in an open topped vessel and then sown onto 4 cms. of moist, sterilized vermiculite (Nitsch and Nitsch used maple sawdust), covered with

a further one cm. and allowed to grow, in total darkness and at 25°C., for 72 hours to 74 hours. At this time the majority of the seedlings had a first internode of approximately 20 to 25 mms. and a coleoptile of 5 to 8 mms.. Plants of these dimensions were used for the bioassay; the coleoptiles were cut off at the first node and sections 4.7 mms. long were cut from the first internode at a position 2 mms. below the first node. The sections were washed, floating on the surface of de-ionized distilled water for one hour and then 10 were transferred to each treatment solution, contained in a 100x 16 mm. glass vial. These were rotated on a horizontal axis, on a disc similar to that described by Nitsch and Nitsch, for 20 hours at approximately 1½ rpm.. After the 20 hour period the length of the sections was measured to the nearest 0.1 mm. using a photographic enlarger set at a 10x magnification. All manipulations except the initial placing of the seed in the washing water and the final measuring were carried out under the green safe-light previously described. This was found to exert a slight phototropic effect; it was therefore shielded down to the lowest level at which the manipulations could be carried out, and the exposure of the plants to it kept to a minimum.

Preparation of the treatment solutions consisted of eluting the appropriate section of the chromatogram into 4 mls. of the buffer used by Nitsch and Nitsch. Elution was carried out for half an hour on a mechanical shaker, and one ml. of the eluate was used for each vial of first internode sections. The remainder of the eluted sample was stored, frozen, in the deep-freeze until used for additional bioassays to obtain

duplicate, or in some cases, triplicate, readings for each sample.

During the course of the work using the two above described techniques, it was found that CCC had an effect on the level of free tryptophan in wheat seedlings. As tryptophan is an amino acid, an attempt was made to make quantitative analyses of samples from the chromatograms using a Beckman/Spinco 120 Amino Acid Analyser. The tryptophan area of the chromatogram was eluted using the Na-citrate standard sample diluter buffer, having a pH of 2.2, and was carried out by using a wick of filter paper dipping into the buffer and allowing it to flow down through the strip of chromatogram attached by surface tension to the opposite end. The first two mls. of eluate were collected and used in the amino acid analyser. As the sample was relatively pure, due to the prior paper chromatography, satisfactory results could be obtained from a run at 50°C., using the short column filled to 18 cms. with Ion Exchange Resin, type 15A, at a pressure of 35 to 40 p.s.i.. Calculations using the results obtained gave the quantity of tryptophan in each sample.

Results

(1) Chlorophyll determinations

(A) Brome grass

Determinations made on the leaves obtained from plants of the first, 'seed'-grown, brome experiment, showed that treatment of the plants with CCC tended to increase the chlorophyll content over that of leaves from untreated plants, as

Table XVI. Effect of CCC on the chlorophyll (a+b) content of brome grass leaves.

		Lbs./a. of CCC. 0	$2\frac{1}{2}$	5	10	20	40	L.S.D. at 5%.
Expt. I.	Mg./g. fresh wt.	2.33	2.24	2.35	2.42	2.50	2.46	-
Expt. II.	Mg./g. dry wt.	14.2	15.3	15.6	15.9	16.6	15.8	1.1
	Mg./leaf	0.56	0.63	0.71	0.65	0.59	0.52	0.14

Data used - mean of 12 replicates.
Expt. I, leaves harvested 5 weeks after spraying.
Expt. II, leaves harvested 8 weeks after spraying.

shown in Table XVI, Expt. I.. These increases were slight and non-significant. The data for chlorophyll content presented here and for all subsequent chlorophyll studies, are the total of chlorophyll a and chlorophyll b per unit of measurement. The quantities of these two chlorophylls were calculated separately but did not show any difference in response to CCC treatment and therefore only the effect of CCC on their total will be discussed. Chlorophyll b, according to the present theory, is probably produced from chlorophyll a (23,47) and thus, unless CCC affected the rate of conversion of chlorophyll a to chlorophyll b, which it apparently did not, no differences in their relative quantities would be expected to occur. It was felt that the lack of a consistent effect of CCC described above might have been due to the apparent genetic variation between plants, and therefore further chlorophyll determinations were made on leaves from brome plants grown from the clonal material.

The results obtained from the second experiment showed a

statistically significant, at the 1 percent level on the basis of mg. of chlorophyll per g. dry weight and at the 5 percent level on the basis of mg. per leaf, increase in chlorophyll content of leaves from CCC-treated plants in comparison with those from the untreated plants, as shown in Table XVI, Expt. II.. On the basis of mg. per g. dry weight there was a trend for increasing chlorophyll content with increasing CCC dose over that of the controls, except at 40 lbs./a. of CCC, at which dose the effect was smaller than that obtained at 20 lbs./a., indicating that the dose of 40 lbs./a. was in excess of that giving optimum results. Calculation of chlorophyll content on the basis of mg. per leaf (calculated from data for dry weight per leaf) showed an apparently larger, but less significant, effect of CCC than that based on mg. per g. dry weight, with a maximum effect at 5 lbs./a. of CCC, in comparison with the previous 20 lbs./a.. The reason for this apparent change in the magnitude of the effect was the differences in dry weight per leaf produced after treatment of the plants with CCC, those at 5 lbs./a. of CCC being heavier than those of the controls, and those at 40 lbs./a. being considerably reduced.

These results indicated that the deeper green colour observed in plants after treatment with CCC can be attributed to increased chlorophyll content, and confirm for brome grass the effects of CCC on chlorophyll content of tobacco leaves published by Humphries (19). Those results for tobacco, however, showed larger differences depending on the basis of the calculations than those observed for brome. CCC, as a continuously supplied soil drench at 10^{-3} M, produced an increase of

only 6½ percent in chlorophyll content per leaf for tobacco compared to the 25 percent increase recorded for brome plants sprayed once with 5 lbs./a. of CCC. On the other hand a 30 percent increase was recorded for the tobacco on a mg. per g. fresh weight basis in comparison with the 16 percent increase for brome on the related mg. per g. dry weight basis obtained for 20 lbs./a. of CCC. Thus, although direct comparison is difficult between the results published for tobacco and those presented above for brome, it would appear that the chlorophyll content of CCC treated plants is increased, following application of the chemical either as a soil drench or as a foliar spray.

(B) Corn seedlings

As the results obtained from brome grass leaves, and those published for tobacco leaves indicated that CCC treatment increased the chlorophyll content, an attempt was made, using dark-grown corn seedlings, to determine the effects of CCC on the rate at which chlorophyll was produced during exposure of these plants to light.

(B I.) Effect of CCC concentration on chlorophyll production.

The first aspect investigated was the effects that different concentrations of CCC might have on chlorophyll production in the corn seedlings. Concentrations of 0, 100, 500, 1,000, 2,500, 5,000 and 10,000 ppm. of CCC were employed. The data obtained for the chlorophyll content of the corn seedlings after exposure to one 24-hour light cycle are presented in

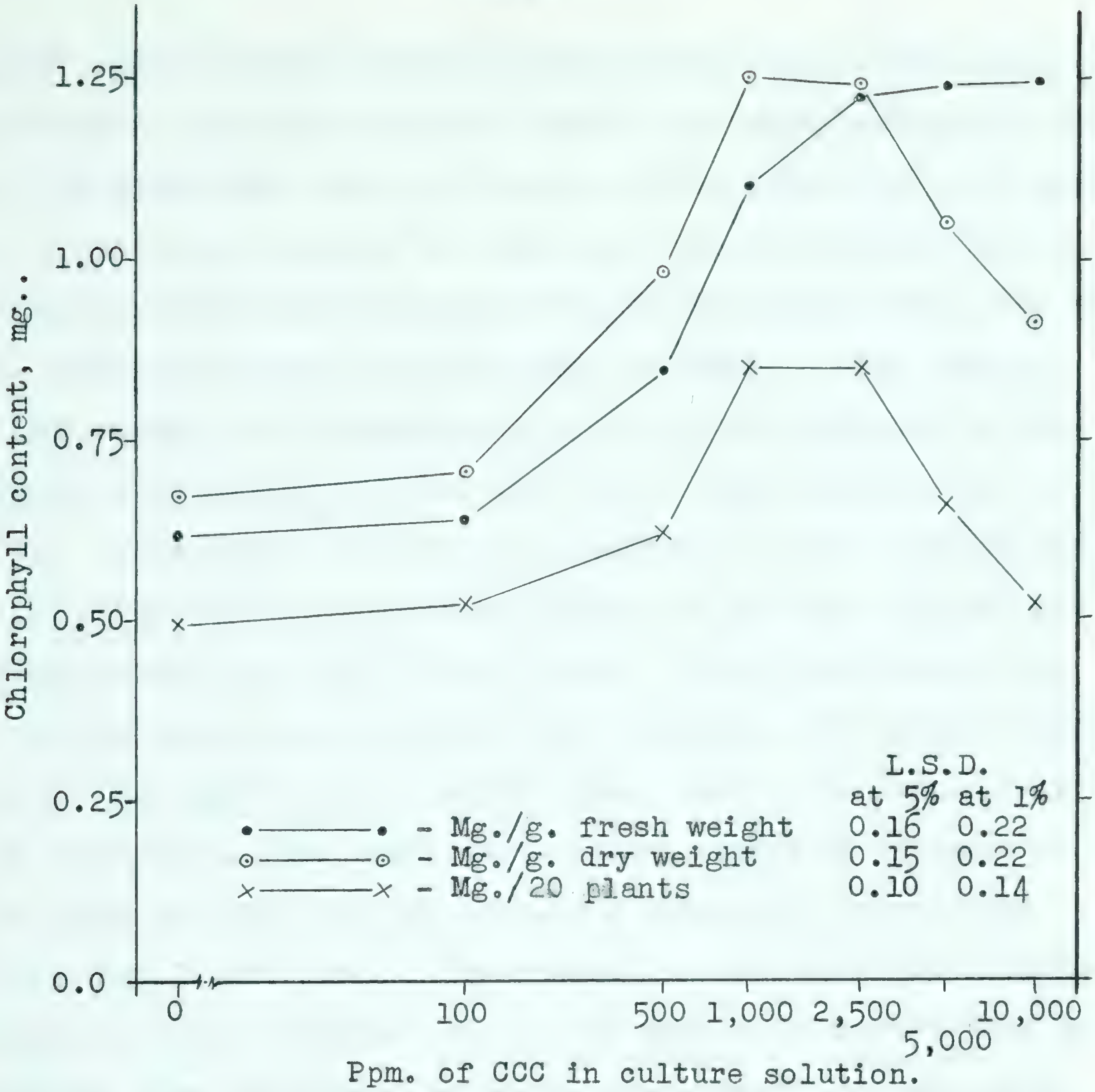


Fig. 26. Effect of CCC on chlorophyll (a+b) production of corn seedlings exposed to light for one 24-hour cycle after being grown in the dark for 14 days.
(Data used - mean of 3 replicates.
data for chlorophyll content as mg./g. fresh weight have been multiplied x 10 for ease of presentation).

Fig. 26.. Chlorophyll content of all plants grown in CCC solutions was higher than that of plants grown in water only, regardless of the bases used for the comparisons. The data obtained for chlorophyll content as mg./plant (calculated from data for plant dry weight) were probably of the greatest significance, and it can be seen from Fig. 26. that the results based on mg. per unit dry weight show a similar trend. The

total amount of chlorophyll produced by a single plant (or equivalent number of plants), rather than that produced by one g. (or other such unit) of fresh or dried material, is of greater significance because it eliminates the possibility that a reduction in volume resulting from CCC treatment could lead to an apparent increase in chlorophyll content. This latter, plant volume, was demonstrated to have some influence on apparent chlorophyll content, but only at the higher levels of CCC. This can be seen if the comparison is made between the data obtained for chlorophyll content on the basis of mg./g. fresh weight and that for mg./plant. The former showed increasing chlorophyll content with increasing CCC concentration up to the highest level, 10,000 ppm., used in the experiment; in comparison, that based on mg./plant showed an increase up to 1,000 to 2,500 ppm. of CCC and a subsequent decrease at 5,000 and 10,000 ppm.. The reason for this apparent discrepancy in results obtained at the two highest concentrations of CCC was that data based on mg./g. fresh weight do not allow for the decrease in plant volume (also in fresh and dry weight) that was recorded for plants grown in those two concentrations, whereas this allowance is made when using data based on mg./plant. The data presented in the remainder of the experiments involving chlorophyll production in corn will, however, show chlorophyll content as both mg./ g. dry weight and mg./plant.

The results of this experiment demonstrated clearly that the inclusion of CCC in the growing medium of corn seedlings increased their ability to produce chlorophyll in comparison with that of plants grown in water only. They also showed that a concentration of 1,000 to 2,500 ppm. of CCC produced the max-

imum increase in chlorophyll content for this species; concentrations of this magnitude were therefore used in the subsequent work.

(B II.) <u>Effect of CCC on protochlorophyll production</u>.

Increased chlorophyll production by plants grown in CCC solution poses many questions concerning the ultimate site of action of the chemical. An experiment was therefore conducted to determine the protochlorophyll production of corn seedlings, in an attempt to discover whether CCC also affected this precursor. Two concentrations of CCC were used for this experiment, 1,000 and 5,000 ppm.; on the basis of the results obtained from the last experiment these were considered sufficient to indicate the effects of CCC on protochlorophyll production. The data in Table XVII. show that corn seedlings grown in CCC solutions developed more protochlorophyll than did the seedlings grown in water only. Although, as would be expected, the amounts present were extremely small, the increase produced by 1,000 ppm. of CCC over that of the controls was of a similar magnitude to that recorded for chlorophyll in the previous

Table XVII. Effect of CCC on protochlorophyll production of corn seedlings exposed to light for one 24-hour cycle after being grown in the dark for 14 days.

		Ppm. of CCC			L.S.D.
		0	1,000	5,000	at 5%
Proto-chlorophyll.	µg./g. dry wt.	27.2	52.5	51.9	11.1
	µg./20 plants	22.4	43.2	35.8	14.4

Data used - mean of 3 replicates.

experiment at this CCC concentration, regardless of the basis used for the comparison. 5,000 ppm. of CCC caused slightly less effect than 1,000 ppm., in agreement with the findings of the earlier experiment.

Thus the results of this experiment indicated that the observed increases in chlorophyll content after growing corn plants in a CCC solution can at least in part be attributed to increased protochlorophyll production. This would suggest that part of the action of CCC on chlorophyll production must be in increasing the rate of metabolism at some point along the pathway leading to protochlorophyll synthesis.

(B III.) Interaction of CCC and ammonium chloride on chlorophyll production.

The possibility that CCC, as it contains the 'trimethylammonium' group in its molecule, was supplying nitrogen to the corn seedlings was considered. In order to obtain data regarding this possibility, plants were grown in a solution containing ammonium chloride, this being chosen because of its molecular similarity to the relevant portion of the CCC molecule, both alone and in addition to CCC. The concentration of CCC used was 1,000 ppm.; calculations showed that 330 ppm. of ammonium chloride would supply approximately the same quantity of nitrogen as would 1,000 ppm. of CCC. The ammonium chloride was therefore used at 330 and 1,000 ppm., the latter concentration being approximately equivalent to 3,000 ppm. of CCC.

The design of the experiment permitted a factorial analysis of the results; this revealed that although both

CCC and ammonium chloride had significant effects on chlorophyll production, as shown in Fig. 27., there was no significant interaction between them. The magnitude of the effect of CCC was similar to that described previously for that concentration. Ammonium chloride at 330 ppm. increased the chlorophyll production of plants grown in water or the CCC solution, the effect of this added ammonium chloride being slightly greater on the former than on the latter. The results presented and discussed here were obtained from the second of two experiments conducted to investigate the effects of CCC in combination with those of ammonium chloride; the

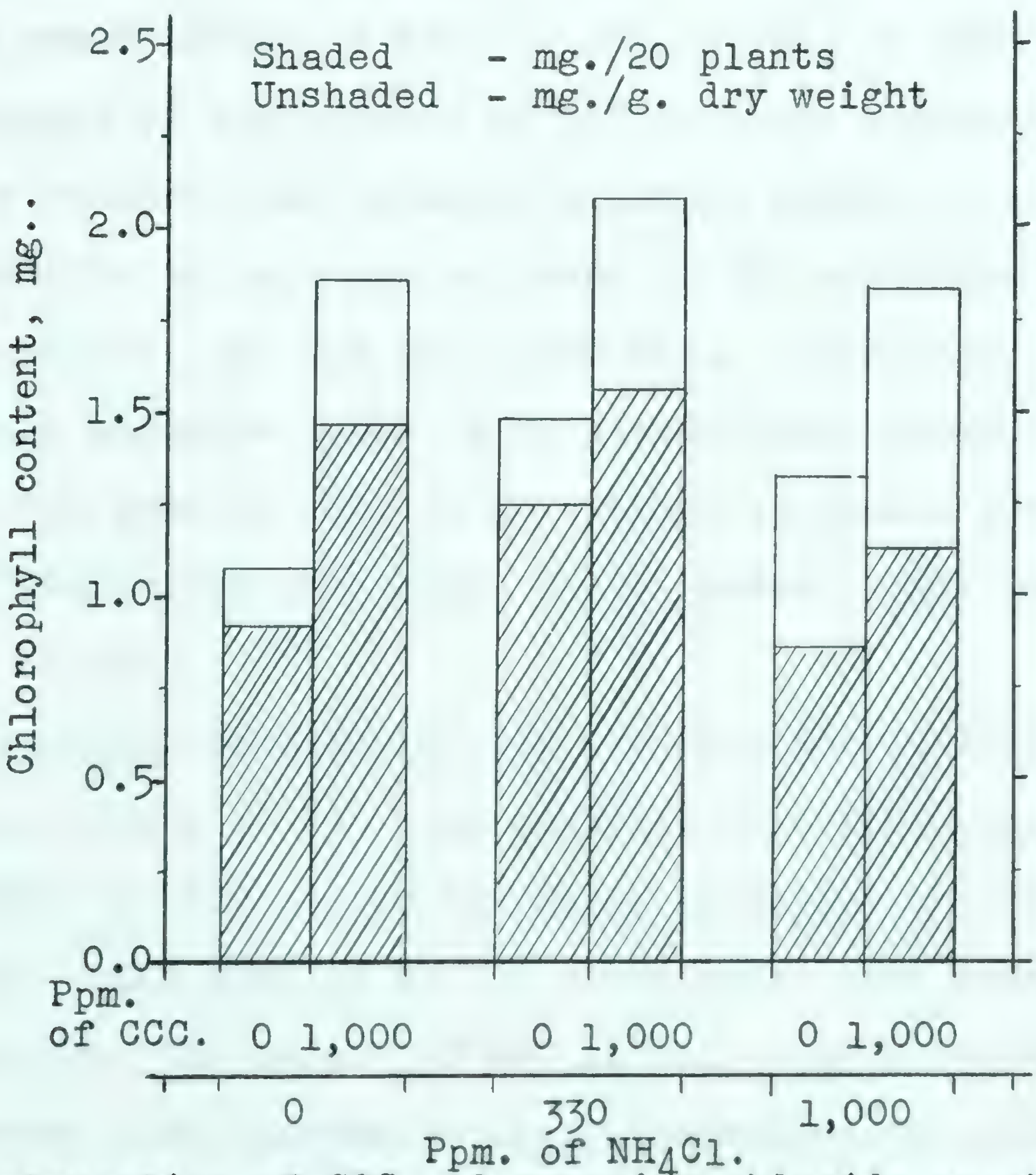


Fig. 27. Interaction of CCC and ammonium chloride on chlorophyll (a+b) production of corn seedlings exposed to light for one 24-hour cycle after being grown in the dark for 14 days.
(Data used - mean of 3 replicates).

results of the first experiment were similar to those obtained for the second.

The results presented above appear to eliminate the possibility that CCC was permitting increased chlorophyll production in the plants by supplying extra nitrogen. In view of the fact that it has been reported (31, Tolbert, personal communication) that CCC is not broken down in the plant, this result was not unexpected.

(B IV.) Interaction of CCC and light intensity on chlorophyll production.

As light intensity appears to be of some importance, although the exact extent of this is not certain at present, in the development of the effects of CCC on plant morphology, the possibility existed that it might exert an effect on chlorophyll production in corn plants grown in CCC solutions. Three light intensities, 15, 190 and 1,800 ft-c., were used for the 24-hour light exposure cycle, with plants being grown in either water or 1,000 ppm. of CCC, in an attempt to obtain information regarding interaction that might occur between light intensity and effect of CCC.

From previous reports (21,53) and from the findings of the earlier experiments it had been expected that there would be a greater effect of CCC at the low light intensity than at the higher ones. The results of the above experiment showed an opposite trend; the effect of CCC on chlorophyll production became greater with increasing light intensity, as shown in Fig. 28.. A factorial analysis of the results revealed that CCC had a highly significant effect (at the 0.1 percent level),

that light intensity had a considerable effect on the plants grown in CCC but had little effect on those grown in water only, and that there was a significant interaction between them (at the 1 percent level). These results therefore indicated that as the light intensity to which the plants were exposed was increased, the difference in chlorophyll production between those growing in CCC and those which were not became significantly greater. This result confirmed the incidental observations made in some of the preliminary experi-

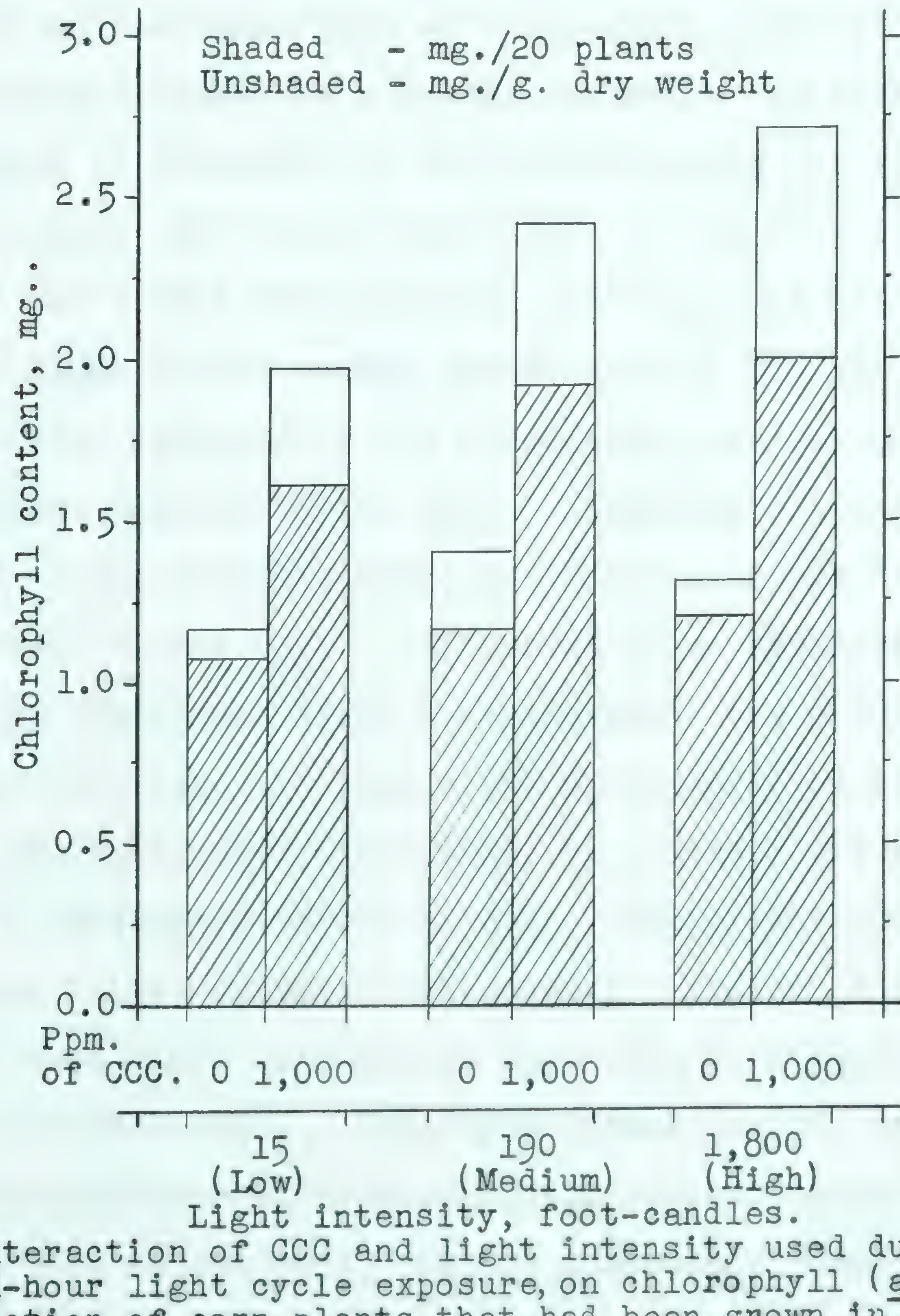


Fig. 28. Interaction of CCC and light intensity used during a 24-hour light cycle exposure, on chlorophyll (a+b) production of corn plants that had been grown in the dark for 14 days.
(Data used - mean of 3 replicates).

ments that had used natural daylight as the light source during exposure, to the effect that the difference in chlorophyll content between plants grown in CCC and those grown in water was less on cloudy than on sunny days. The possible implications of this result will be discussed later.

(B V.) Effect of CCC on the rate of loss of chlorophyll.

The possiblity that the increased chlorophyll content in plants grown in CCC solutions could, at least in part, be attributed to an increased rate of chlorophyll production has already been considered to a certain extent. An experiment was conducted to determine if CCC also affected the rate at which chlorophyll was broken down after it had been produced. To do this the plants were grown in 1,000 ppm. of CCC or water, exposed to light in the normal manner and, at the end of the exposure cycle, returned to the germinating cabinet and allowed to continue growing in the dark. Sampling was carried out at the end of the exposure cycle and after 2, 4 and 8 days of further growth in the dark. It proved to be impossible to continue the experiment after 8 days because the plants, then three weeks old, began to show signs of disease and degredation.

The results of this experiment, as presented in Fig. 29., revealed an unexpected effect of CCC. The plants grown in water showed little change in chlorophyll content during the first four days after exposure to light and a subsequent decline over the last four days. The plants grown in CCC, however, continued to increase their chlorophyll content during the first four days in the dark, with a subsequent decrease

occurring during the last four days. Thus the maximum differences between plants grown in CCC and those grown in water developed after four days growth in the dark after exposure to light; the relatively small difference recorded at the end of the light exposure cycle can possibly be attributed to the relatively low light intensity used for this purpose. The rate of loss of chlorophyll was slightly higher during the last four days of the experiment for the plants grown in CCC than for those grown in water, probably because the treated

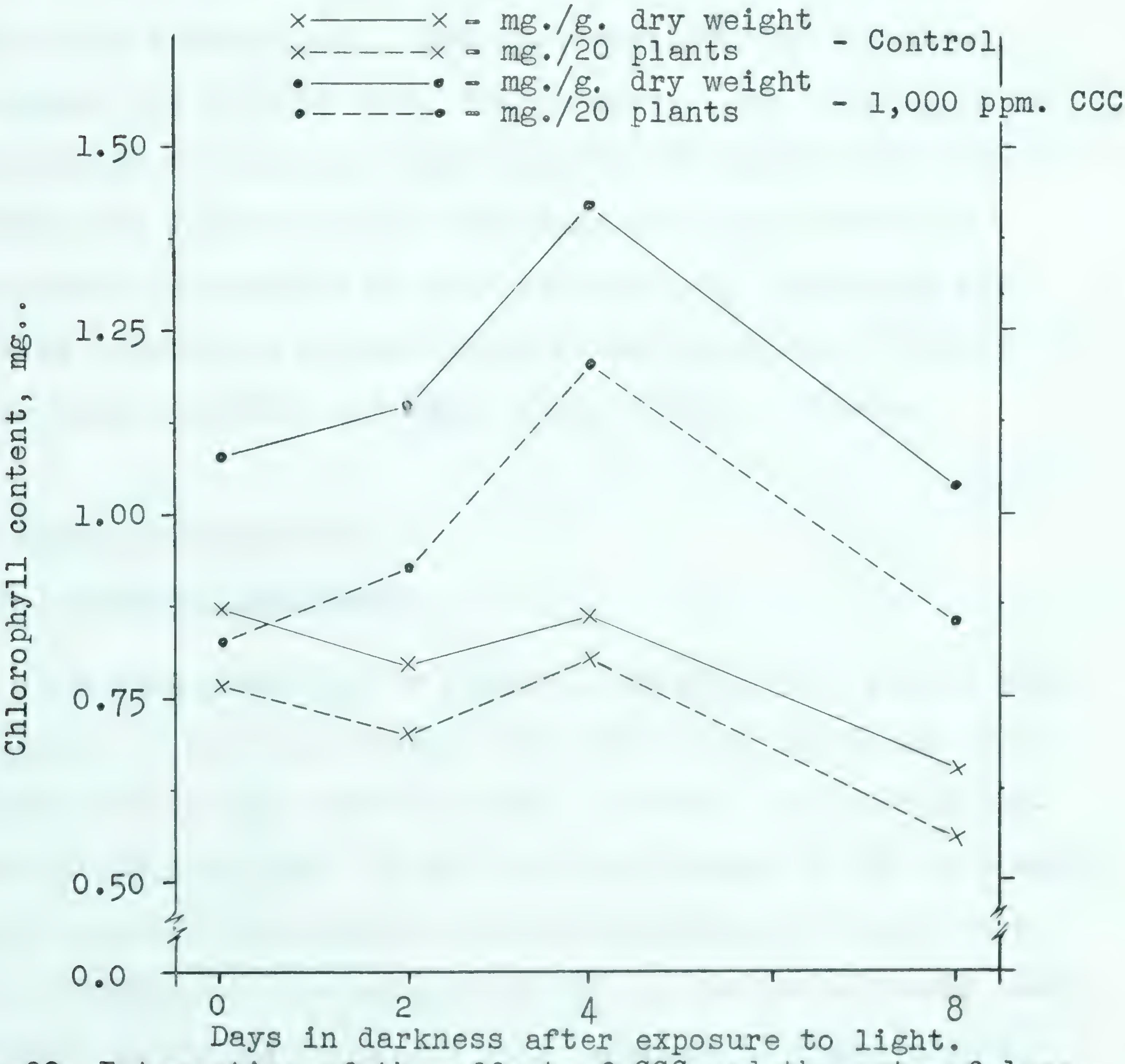


Fig. 29. Interaction of the effect of CCC and the rate of loss, in the dark, of chlorophyll (a+b) in corn seedlings exposed to light for one 24-hour light cycle after being grown in the dark for 14 days.
(Data used - mean of 3 replicates).

plants had a greater initial quantity of chlorophyll to lose, rather than because of an actual difference in the rate of degradation.

The results of this experiment are interpreted to mean that CCC had little effect on the rate of loss of chlorophyll of plants grown in the dark. The plants grown in a CCC solution, however, appeared to continue developing chlorophyll in the dark for several days after exposure to light in comparison with the plants not grown in CCC which did not possess this ability, the chlorophyll content remaining constant during the same period. The fact that CCC did not appear to affect the rate of loss, but to enhance the production of chlorophyll in the dark after exposure to light, tends to confirm the suggestion that CCC must exert its effect on chlorophyll production in corn seedlings by increasing the rate of metabolism at some point along the pathway leading to protochlorophyll, and hence chlorophyll, synthesis.

(2) Auxin determinations

(2.a.) Initial experiments.

The data published by Kuraishi and Muir (26) showed that treatment of pea plants with CCC reduced the amount of diffusible IAA in the stem to a very low level, and as the suggestion has been made (22,32) that the action of CCC on plants may be through the native gibberellin system, attempts were made to determine the effects of CCC on the native auxin level in grass seedlings to obtain data relevant to these topics.

Descriptions of the plants used for this section of the

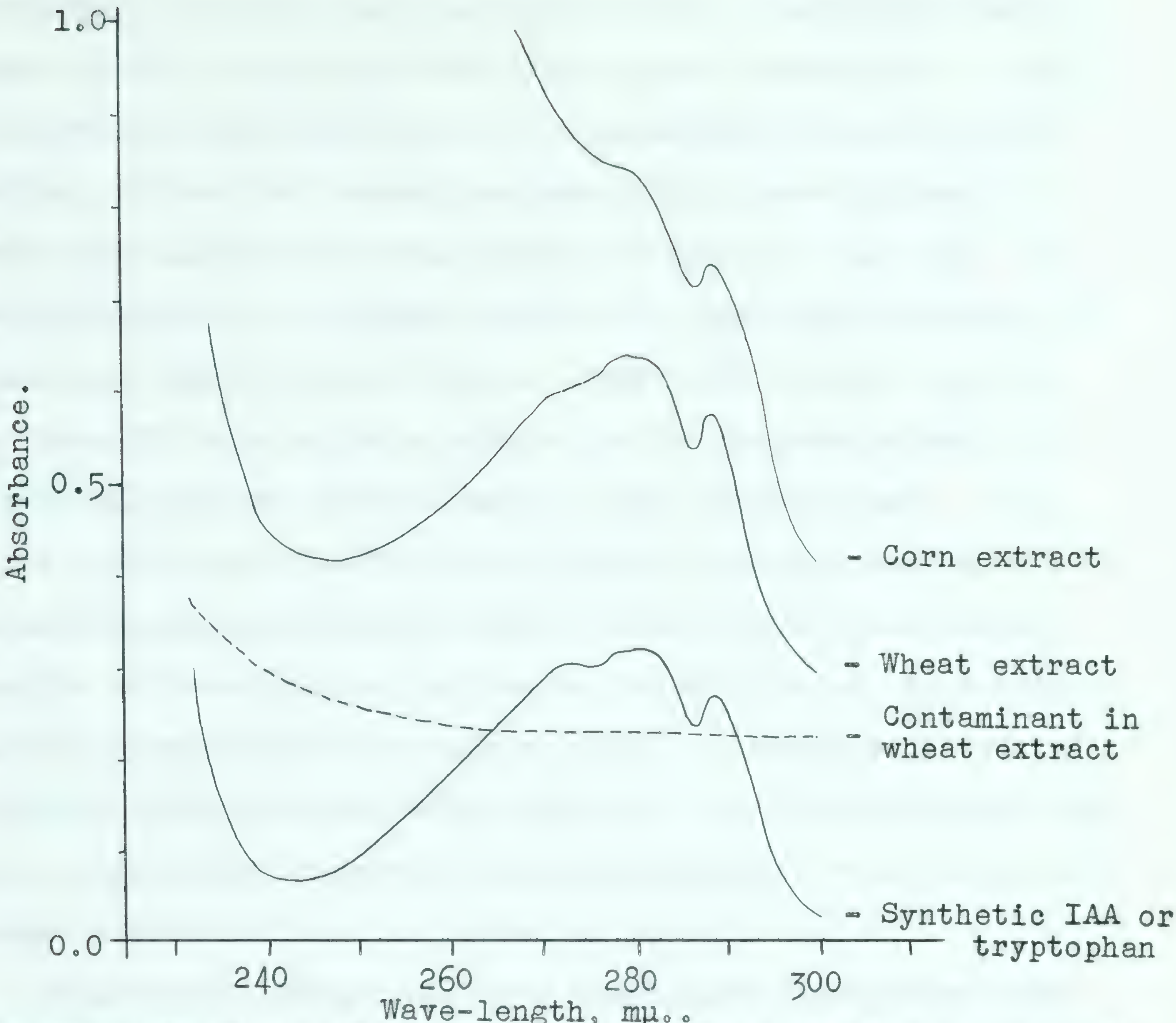


Fig. 30. Comparison of the absorption curves of indole compounds from plant extracts with those of synthetic IAA and L-tryptophan using a recording spectrophotometer.

work have been made previously. Corn seedlings were chosen for the initial work because it had been reported (see Leopold (29)) that immature corn kernels have a high IAA content, and it was considered possible that the young seedlings might therefore also contain large quantities of this auxin. The solvent used for the chromatographic separation was isopropanol:ammonia (28 percent):water (IP:A:W) at the ratio of 8:1:1 (w/v). Spraying the chromatogram with DMAC revealed a large spot containing an indole compound at an Rf of 0.4, approximately that of

synthetic IAA under the same conditions. Spectrophotometric assay of the eluate from this area proved impossible, as the absorption curve produced over the measuring range of 300 to 240 mμ. did not correspond accurately with that typical of synthetic IAA over the same range, as shown in Fig. 30. It was concluded that another compound (or compounds) capable of absorbing light of wave-lengths between 300 and 240 mμ. had the same Rf value as this unknown indole compound when 8:1:1 IP:A:W solvent was used to develop the chromatograms. The use of different IP:A:W ratios, butanol:methanol:water, butanol:ammonia:water and carbon tetrachloride as chromatographic solvents did not effect any separation of the contaminating compound and the unknown indole. Extraction of the original plant material with both water and acetone as solvents gave equally poor results, and partitioning of the extract over carbon tetrachloride also proved to be of no value.

Therefore, without the more complicated acid-ether type of extraction and purification, possibly followed by two dimensional chromatography, recovery and assay of indoles in corn using the described spectrophotometric technique does not appear possible. Attempts were made therefore to estimate the auxin level in oat seedlings, as this species is frequently used as the material for bioassays. The recovery of an indole compound from the extract obtained from oats, at an Rf value corresponding closely to that of synthetic IAA and with an absorption curve similar to that of synthetic IAA in a spectrophotometer, was effected without difficulty. It was during the course of this work, however, that it was observed

that oat seedlings did not appear to be much affected by CCC; plants grown in 1,000 ppm. of CCC showed no reduction in height in comparison with those grown in water (28.8 cms./plant compared to 27.2 cms./plant respectively). Subsequent assay of the unknown indole compound obtained from these seedlings showed that there was no difference in its level between plants grown in CCC and those in water. Four replicates, of concentrations of 0, 100 and 1,000 ppm. of CCC, all showed a similar lack of effect of CCC.

For the purpose of determining the effects of CCC on the auxin level of treated plants, oats therefore proved to be of limited value and attempts were then made to obtain data relevant to this effect using wheat seedlings. Wheat was finally chosen because the effects of CCC on it have already been investigated (53); it was considered that data concerning the auxin content of this species would be valuable. An indole compound was extracted and detected with DMAC without difficulty, but the absorption curve obtained between wave-lengths of 300 to 240 mμ. showed characteristics similar to those for the indole obtained from corn. The use of 7:1:2 IP:A:W instead of 8:1:1 improved the characteristics of the curve obtained to the extent that although the absorbance was high throughout the 300 to 240 mμ. range, the pattern of the curve closely resembled that of synthetic IAA, as can be seen from Fig. 30.. It appears likely that a contaminant having the same Rf value as the indole compound was present in the wheat extract, but that its absorbance must have been increasing only slightly as the wave-length used decreased; the dotted

line in Fig. 30. represents the theoretical possible absorbance of this unidentified compound. In order to obtain data on the actual amount of the indole compound that was present, the difference between the reading obtained at 300 mμ. and that at 280 mμ. was used; this proved to give relatively reliable data.

The Rf value of synthetic IAA was determined using the 7:1:2 ratio of IP:A:W as the chromatographic solvent, and was found to be 0.60 to 0.62; that of the DMAC-located indole compound from wheat was found to be 0.56 to 0.58 and the compound was assumed, erroneously, to be IAA. Measurements were made on the extracts from wheat plants grown in an increasing concentration range of CCC, the experiment being duplicated.

In an attempt to confirm the data obtained using the spectrophotometric method, a bioassay was conducted on extracts from the same Rf region, supposedly that of IAA. It was immediately obvious that this area, at a concentration calculated from the spectrophotometric data to be biologically active, was not. Therefore it had to be concluded that the indole compound thought to be IAA was in fact another indole compound. An extensive check was made on the indole compounds that could be detected on chromatograms by using several different spray reagents and by obtaining Rf values for several synthetic indole compounds. Spraying the chromatograms with DMAC, as previously described, revealed a compound at Rf 0.57 which turned blue-purple and a second band at Rf 0.46 was also apparent, as a pale blue colour which faded rapidly after spraying (Fig. 31, nos. 3 and 4). The application of synthetic

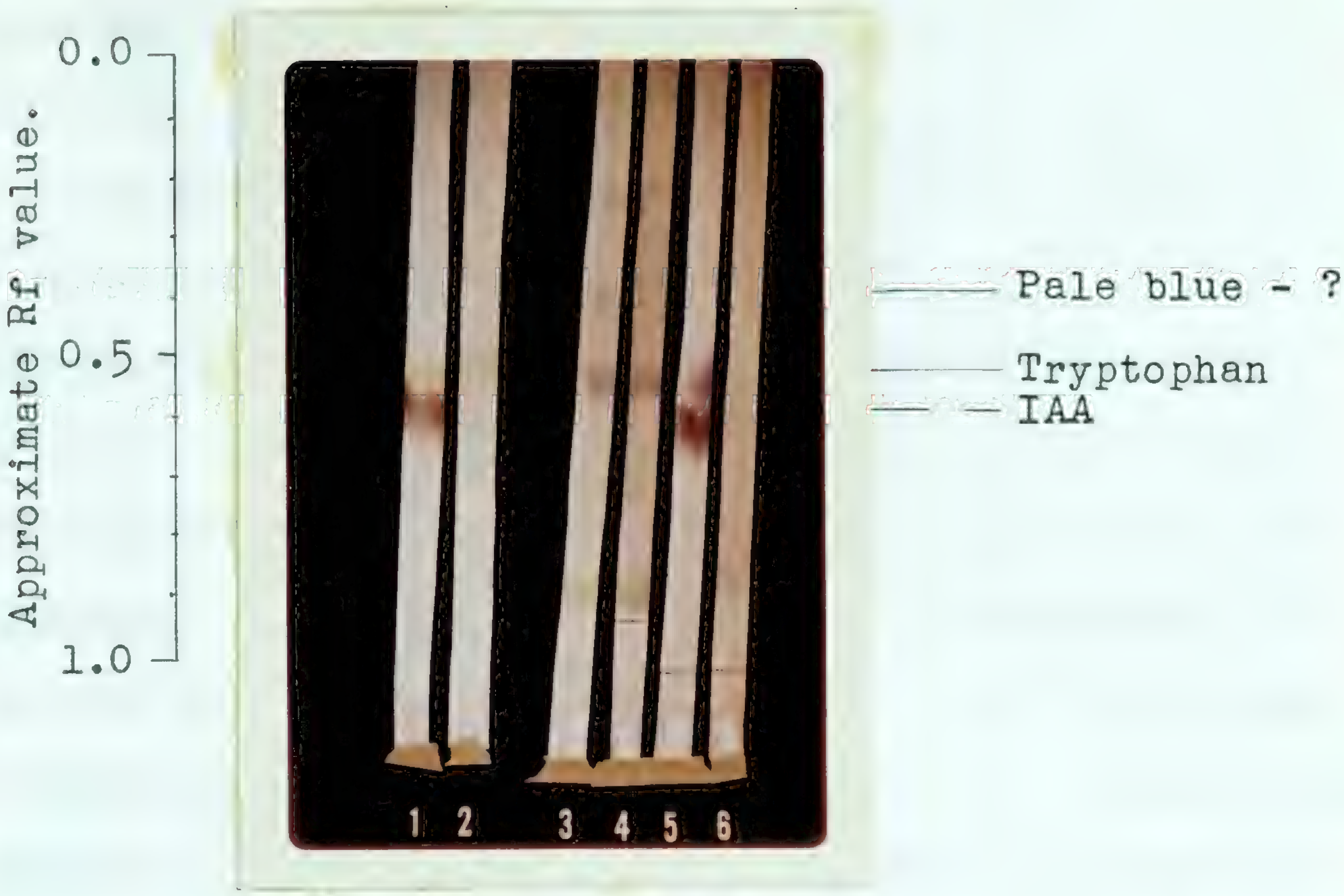


Fig. 31. Chromatogram strips developed in 7:1:2 IP:A:W and sprayed with DMAC and nitrous acid to show location of wheat indoles in relation to synthetic IAA and tryptophan. (For explanation of this figure, see text; numbers used for reference to individual strips).

IAA to the start line of the chromatogram in addition to the plant extract revealed a red-purple band at Rf 0.61 after spraying with DMAC, slightly ahead of the unknown indole compound at Rf 0.57. By checking the Rf values of several indoles it was found that L-tryptophan had an Rf value of 0.57 under the conditions used during the development of the chromatograms and possessed the same blue-purple colour of the unknown indole compound, in contrast with the red-purple colour developed by synthetic IAA; this difference can be seen clearly in Fig. 31, on strip no. 5.. The following indole compounds were also tested; indoleacetonitrile, indoleacetaldehyde and indolepropionic acid, but all had Rf. values completely different from those of either of the DMAC sensitive unknown indoles obtained from wheat. A mixture of synthetic IAA and L-trypt-

ophan were just separated by the 7:1:2 IP:A:W solvent, at Rf values of 0.57 and 0.61 respectively, as shown in Fig. 31., no. 5.. The chromatograms of plant extract and those of a mixture of synthetic IAA and L-tryptophan were also sprayed with the reagents DMAB, Salkowski reagent and nitrous acid (Fig. 31., no. 1 for the two synthetics, no. 2 for the plant extract), all confirming that the unknown indole at Rf 0.57 was tryptophan. Nitrous acid proved extremely valuable because, as can be seen from Fig. 31., nos. 1 and 2, IAA turns red and tryptophan yellow with this reagent; the unknown wheat indole compound turned yellow, again indicating that it was tryptophan. As a further check, a set of chromatograms, identical to those described above, were developed in a 70 percent

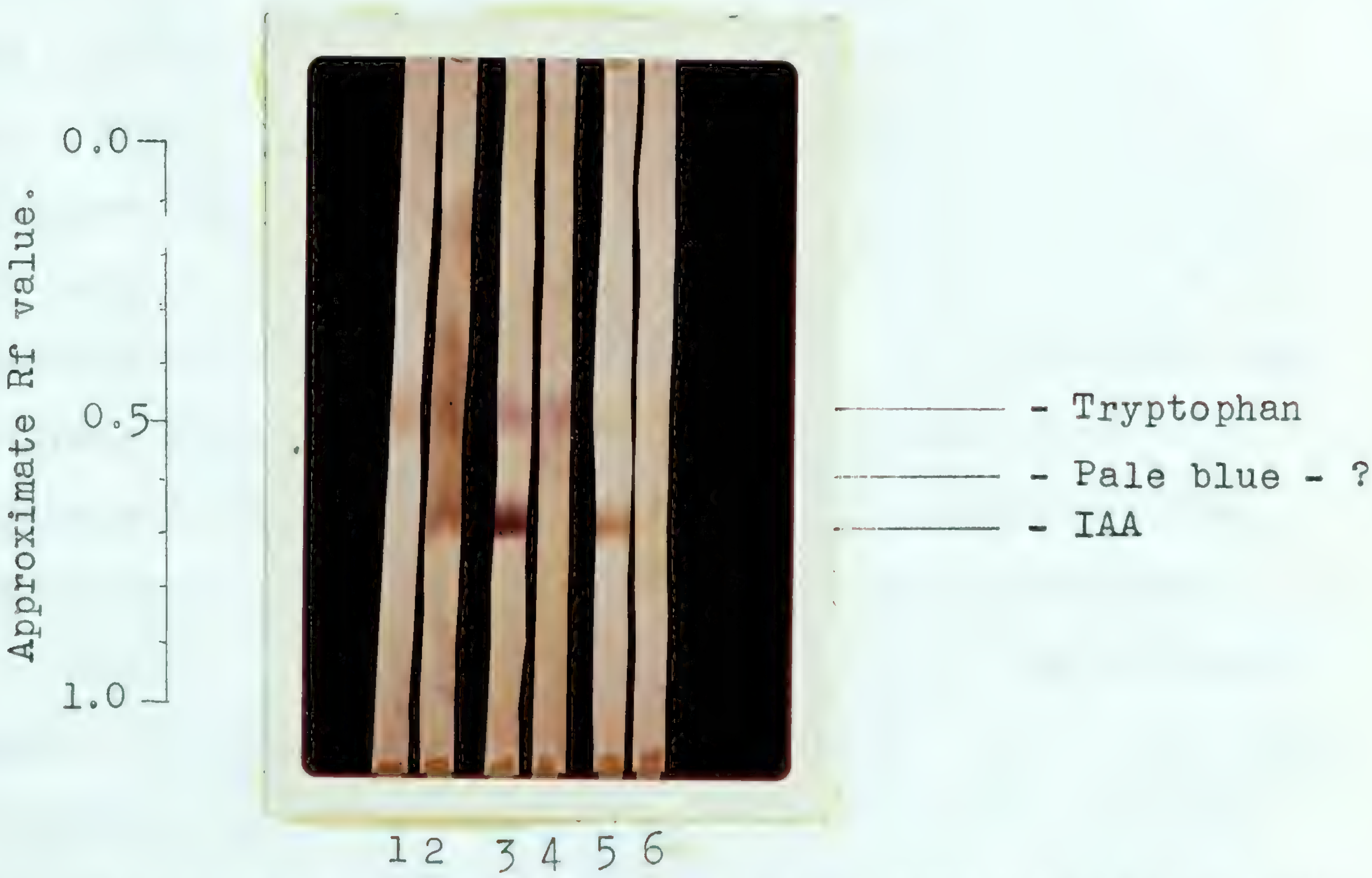


Fig. 32. Chromatogram strips developed in 70 percent ethanol and sprayed with DMAC, Salkowski reagent, nitrous acid and ninhydrin to show the location of wheat indoles in relation to synthetic IAA and tryptophan.
(For explanation of this figure, see text; numbers used for reference to individual strips).

ethanol solvent system. DMAC spraying revealed that the blue-purple indole compound that had been at Rf 0.57 in the IP:A:W solvent had an Rf value of 0.51 and that the pale blue previously at Rf 0.46 was at Rf 0.59, as shown in Fig. 32., no 4.. L-tryptophan was found to have an Rf of 0.51 and IAA one of 0.80 (Fig. 32., nos. 3 and 5). The locations of these compounds on the chromatogram were again confirmed by nitrous acid (Fig. 32., no. 6 for wheat extract, no. 5 for synthetics) and Salkowski reagent (Fig. 32., no. 1, for wheat extract only). Thus it appeared clear that the unknown indole compound recovered from wheat seedlings was tryptophan. Tryptophan is an amino acid and gives a positive colour reaction with ninhydrin; a chromatogram developed with 70 percent ethanol was therefore sprayed with this reagent, prepared according to Block (2) and although a ninhydrin-positive reaction occurred from Rfs 0.16 to 0.75, a darker band developed at 0.51, corresponding to that of tryptophan (Fig. 32., no. 2). The final conclusive evidence that the unknown indole compound was tryptophan was supplied by data obtained using an amino acid analyser. In preparation for this analysis a known quantity of L-tryptophan was added to a sample obtained from the area of a chromatogram identified as that containing tryptophan by the above described techniques. A single peak was obtained at the correct position for synthetic tryptophan, but only approximately half of the quantity calculated to be present from the data provided by this peak could be accounted for by the known added amount of synthetic L-tryptophan, the remainder therefore being from the wheat extract. It was concluded that a large quantity of tryptophan was being

extracted from wheat seedlings; the previously recorded data, first thought to represent IAA, were compared with calibration data for L-tryptophan in a spectrophotometer, and the quantity present in the samples was determined. It should be added that the type of absorption curve obtained in the spectrophotometer over the 300 to 240 mμ. wave-length range for synthetic L-tryptophan is in all respects similar to that of synthetic IAA.

(2 b.) Determination of tryptophan content of wheat seedlings.

Measurements of the tryptophan content of wheat seedlings using the spectrophotometric method were performed on plants grown in water or CCC concentrations of 100, 500, 1,000 and 2,500 ppm.. The results in Table XVIII show that there was a significant trend for lowered tryptophan content as the CCC concentration increased. The maximum reduction in tryptophan content occurred at the highest CCC rate, that of 2,500 ppm., and amounted to 35 percent. The amount recovered appeared to vary somewhat from experiment to experiment, the quantities

Table XVIII. Effect of CCC on the level of 'free' tryptophan in 11-day old dark-grown wheat seedlings.

		Ppm. of CCC.					L.S.D.	
		0	100	500	1,000	2,500	at 5%	at 1%
Spectro-photometer	Expt. I.	53.4	42.0	44.4	39.3	36.6	7.3	10.3
	Expt. II.	98.2	-	82.1	73.4	56.8	13.0	19.6
Amino acid analyser.		123.4	-	114.6	-	80.3	38.2	-

Data used - mean of 4 replicates - Tryptophan, μg./0.25 g. dry weight.
Spectrophotometer data - mean of 2 readings per sample.

obtained in Expt. II being approximately twice as large as those of Expt. I; the differences between plants grown in CCC and those which were not, however, remained of the same relative magnitude. The data obtained by the amino acid analyser method were only for water and two concentrations of CCC, 500 and 2,500 ppm., because of the length of time involved in obtaining a single reading by this method, and are presented in the corresponding section of Table XVIII. These results confirmed that tryptophan was reduced by CCC treatment, the effect at 2,500 ppm. of CCC being slightly greater than a one third loss in comparison with the controls; this effect was of the same order of magnitude as that obtained using the spectrophotometric measuring technique. The amount of tryptophan recovered, however, was considerably greater; no explanation can be given for this unless the previously mentioned variation between experiments was sufficiently large to encompass the observed differences or that the pH 2.2 sample diluter buffer used for eluting the tryptophan from the chromatogram was able to recover a higher percentage of it from the paper. This latter possibility, however, seems unlikely, as, although no check was made for tryptophan, about 98 percent of synthetic IAA was found to be recovered from the chromatogram paper with either water or methanol as the elution solvent. The possibility that the apparent decrease in tryptophan content after treatment of the plants with CCC was due to a lack of translocation from the seed to the seedling was considered. An experiment was conducted to investigate this using samples with the old seeds intact and others from which the old seeds had been removed; no differences could be detected between such

samples, CCC-treated or not. It should be pointed out that the tryptophan measured by the above described techniques was that extractable from homogenized tissue using ice-cold methanol and can therefore probably be considered to be 'free' or unbound tryptophan.

Thus these results showed that the 'free' tryptophan content of wheat seedlings grown in a CCC solution was lower than that of plants grown in water only. The possible implications of this on the growth of CCC treated plants are manifest, and will be discussed later.

(2 c.) Determination of IAA level in wheat seedlings.

As the DMAC-positive indole compound at Rf 0.56 using the 7:1:2 IP:A:W chromatographic solvent proved to be tryptophan, it was realised that IAA, if present, was at a level below that detectable with DMAC. In order to determine if CCC had any effect on the growth promoting indoles as well as on tryptophan a series of bioassays were conducted. The various regions of chromatograms developed in IP:A:W that possessed biological activity were determined by cutting the chromatogram into sections representing divisions equivalent to 0.1 Rf units, and subjecting the eluate from each section to bioassay. The results, as presented in Fig. 33, showed that there were three main areas of biological activity. Slight activity was recorded at the origin and no activity was observed between Rf values 0.1 to 0.4 inclusive and also 0.9 to 1.0. A moderate elongation of the oat first internode sections occurred between Rf 0.4 to 0.5, corresponding to the pale blue area observed

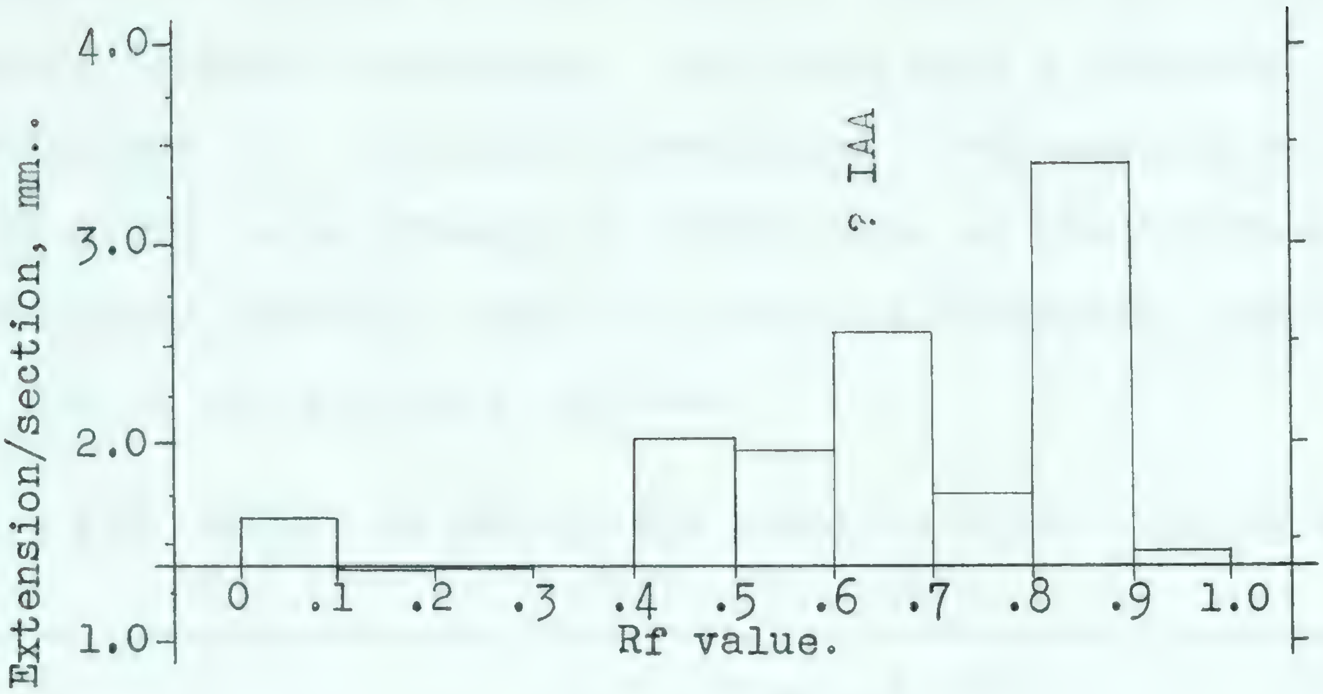


Fig. 33. Oat first internode bioassay of growth activity regions of a chromatogram of wheat extract, developed in isopropanol:ammonia:water at 7:1:2 (w/v).
(Data used - mean of 2 chromatograms).

after spraying the chromatogram with DMAC. Elongation from the section between Rf 0.6 and 0.7 was somewhat greater than that of the pale blue area, and corresponds to the Rf value of synthetic IAA. The greatest elongation was recorded for the section between Rf 0.8 to 0.9; the identity of this compound was not determined, and it did not give a colour reaction with DMAC. The elongation observed for the sections between Rf 0.5 to 0.6 and 0.7 to 0.8 was probably due to 'trailing' of the compounds producing elongation in the sections from Rf 0.5 to 0.6 and 0.8 to 0.9 respectively. Subsequent experiments revealed that the growth promoting activity obtained from substances present in sections from Rf 0.4 to 0.5 and 0.8 to 0.9 was extremely erratic, and in some cases appeared to be non-existent, only that of the section from Rf 0.6 to 0.7 having a consistent effect. Since this corresponded to

the Rf value of synthetic IAA it was used for the subsequent bioassay attempting to determine the effect of CCC on auxin content of wheat seedlings. For this work a slightly larger section was cut from the chromatograms, representing Rf 0.58 to Rf 0.70, in an attempt to retain more of the IAA that might be trailing, assuming that the observed biological activity was due to the presence of IAA.

Table XIX. Effect of CCC on the level of 'free' IAA in 11-day old dark-grown wheat seedlings.

		Ppm. of CCC.					L.S.D.
		0	100	500	1,000	2,500	at 5%
Solvent.	IP:A:W.	39.0	32.1	29.6	23.4	20.9	11.4
	70% ethanol.	20.5	8.1	8.8	11.2	6.7	-

Data used - IAA as µg./litre; mean of 4 replicates. each replication mean of 3 bioassays.

The results of measurements of the IAA content made on plants grown in water or CCC at concentrations of 100, 500, 1,000 and 2,500 ppm. are presented in Table XIX., and show that as the CCC concentration increased the IAA content decreased. The results obtained from chromatograms developed with IP:A:W showed a more or less consistent, and significant, trend for reduced IAA content, plants grown in 2,500 ppm. of CCC showing the largest reduction and having approximately one half the IAA content of those grown in water only. The Rf region equivalent to that of synthetic IAA cut from chromatograms developed in 70 percent ethanol showed considerably more variable results, although all plants grown in CCC had less IAA

than those grown in water. The reason for the more variable results, and generally lower yields, obtained using ethanol was probably that the development of the chromatograms only took 12 hours in comparison with the 18 hours necessary when IP:A:W was used as the solvent. This resulted in considerably more diffuse spots, which were more difficult to remove completely and accurately from the chromatogram prior to elution. It should be pointed out that the material used for both the above described bioassays was obtained from the same plant extracts, and that the amino acid analyser measurements of tryptophan content were made from the same IP:A:W developed chromatograms that were used for the above bioassay.

It can be seen from the results presented in Table XIX that the actual quantity of IAA per sample was very low. A reading of 40 µg. of IAA per litre represents a quantity of approximately 0.175 µg. of IAA per 0.25 g. of wheat seedlings (dry weight), which in turn is equivalent to about 13 plants. As the Rf value of the above described compound corresponded with that of synthetic IAA and because it possessed growth promoting activity it was assumed that IAA was being measured. Thus, the above described results confirmed those of Kuraishi and Muir (26), although the magnitude of the effect was not as great as that which they reported for peas. The implications of the results presented above will be discussed later.

DISCUSSION AND CONCLUSIONS

Results presented in the foregoing sections of this treatise represent a variety of effects of CCC on grasses. Some of these results, for several species of grasses, have paralleled effects which had previously been described only for other plants, and some concerned effects that have so far received little attention or which had not been previously reported.

In most of the previously published work, CCC was applied as soil drenches or in nutrient solutions. Throughout the work presented in this treatise, except for the plants grown for use in the physiological studies, foliar spray techniques were used to apply the CCC. It is possible that if soil drenches had been used their effects would have been greater than those resulting from spraying. The results recorded after spraying wheat with CCC were, however, equal to, or greater than, those previously reported for this species after CCC was applied as a soil drench (53). It appears, therefore, that foliar sprays of CCC are effective when used on grasses, and that the treatment of large areas of grass sward would be feasible, if a field use for CCC is discovered.

The general tentative conclusion from the work done here is that CCC applied to grasses growing under field conditions had no useful effect. Inherent differences in soil and vegetation may account for some of the differences in yield observed under field conditions at the University of Alberta at Kinsella, and, as previously discussed, differences in rainfall could also account for some of the observed discrepancies

in the results obtained in different years. An apparent lack of effect of CCC on drought resistance was recorded for creeping red fescue grown under greenhouse conditions. This result seems to refute the above argument. It is felt, however, that the apparent lack of effect may have been an artefact of technique, such as the ratio of plant surface to soil surface, the type of soil used or the amount of CCC applied, rather than a lack of effect on the grass. Thus the response of CCC-treated grasses to drought is uncertain. The possible effect of CCC on the tolerance of plants to salt concentration (previously discussed) could therefore have been the more important factor. This apparent anomaly between the effect of CCC in different years at Kinsella cannot therefore be resolved by the data presented in this treatise. The dry matter yields obtained in the greenhouse experiments, except with regard to the winter grown Kentucky bluegrass, tend to support the conclusion that spraying grasses with CCC does not, under average Alberta summer conditions, produce any beneficial changes in dry matter yield. Some of the greenhouse experiments do suggest that spraying grasses with CCC prior to cutting may increase their ability to produce regrowth. This would be of practical value in areas where a second-cut hay crop cannot normally be obtained because of insufficiently rapid regrowth after the first cutting. Considerable further experimentation would be required, however, before any definite conclusions could be made concerning this potential aspect of the effects of CCC.

Visible effects of CCC on morphology of grasses in the current research were similar to those reported in other work

with certain grasses and some other species of plants. Treatment with CCC produced shorter plants, which usually possessed darker green leaves and in some cases had larger numbers of tillers and leaves than the controls. The dry matter yield was not significantly affected , except for the increases recorded for the winter grown Kentucky bluegrass, but there was a trend for increased root:shoot ratio. No phytotoxicity was observed, except for brome plants that had poorly developed roots at the time of spraying, even when using the extreme dose of 40 lbs./a. of CCC or the high concentration of 10,000 ppm. in the growing medium.

The effects of CCC, or any other growth regulating compound, on the morphological development of individually grown plants may not be identical with the effects on plants of the same species grown under field conditions, since the requirements for light, moisture, etc. are different in the single plant-per-pot experiment. An example of how significant this could be can be demonstrated theoretically for any well developed crop. If crop plants reach a stage of growth such that when a new leaf is produced its respiration rate is higher than its rate of photosynthesis, due to mutual shading of the leaves, or any other factor, then the relative growth rate of the individual plant will decline. If a chemical such as CCC is applied at such a stage of crop development it might increase the leaf number per plant and yet decrease the dry matter yield. Humphries (19) has already suggested that shortening of the internodes of mustard plants after treatment with CCC might lead to increased mutual shading of the leaves,

which could explain the lowered dry matter yield that was recorded. It seems appropriate to mention these factors while considering the effects of CCC on the morphological characteristics of individual grass species grown in pots under greenhouse conditions. It also offers a possible explanation for the discrepancy between some of the results obtained in the greenhouse and those obtained under field conditions.

Height was reduced in all plants by treatment with CCC, the magnitude of the effect increasing with increasing rate of CCC application. A maximum effect was attained at about four to six weeks after spraying; this was followed by a subsequent decline, and the CCC treated plants frequently appeared to be elongating faster than the controls towards the end of the experiments. This could be due to destruction of CCC in the plant or to dilution as the plant volume increased, eventually reaching a concentration of CCC at which no inhibition of elongation could be effected. In regard to brome grass, an actual increase in elongation was observed when using CCC at low concentration; this was in agreement with the reported effects of the closely related compound AMAB on *Ulothrix subtilissima* (7), in which species low concentrations of AMAB caused growth promotion. The effect of CCC on plant height has been attributed to a reduction in internode elongation (e.g., 3,19,26,53,59) and this conclusion is supported by the data obtained for the majority of the work reported in this treatise, but it does not explain the reduction in height observed for creeping red fescue or that of corn and wheat seedlings grown for the physiological studies. In the latter

two instances virtually no stem elongation occurred in either treated or untreated plants, the data recorded for height consisting mainly of leaf length. Measurements of brome grass leaf blade length, in contrast to the length of leaf blade plus leaf sheath, showed no significant effect of CCC. If the same is true for the creeping red fescue and the corn and wheat seedlings, then it would appear that CCC reduces the elongation of leaf sheaths in addition to that of internodes in grasses.

Although the statement has been made that CCC decreased the height of all species investigated, the magnitude of this effect varied from one species to another. Species with long stems were affected to a much greater extent than those which normally have shorter stems. This difference in effect is almost certainly due to inherent differences in the habit of growth of the species. Differences in response were also recorded for wheat, oats and barley, all relatively long stemmed species. Oats showed only slight effects in comparison with the severe reduction in height recorded for both wheat and barley. It is possible that this type of difference in the response to CCC can be attributed to physiological differences between the species, such as in the inherent level of 'free' tryptophan and/or IAA. The latter possibility will be discussed later.

In all the previously published data no reference has been made to the effects of CCC on an aspect of plant morphology closely related to stem length, namely rhizome production. It was found that the effect of CCC on rhizome pro-

duction was essentially similar to that on the stem, though it was more pronounced. Plants treated with CCC produced shorter rhizomes, the magnitude of the effect increasing as the dose of CCC applied was increased. The dry weight of rhizome per cm. of CCC-treated plants was increased over that of the controls. This corresponds to findings for stem diameter after treating plants with CCC. Decreased rhizome production for plants such as brome grass, which relies largely on rhizomes for the production of secondary vegetative growth, was probably the reason for the observed decrease in total dry matter content.

As described previously, leaf production per apical meristem was not affected by treatment of the plants with CCC. However, as the number of tillers appeared to be increased, the total leaf number per plant was greater for plants receiving CCC than for plants which did not. Tiller production was increased by treatment of the plants with CCC, except at the highest doses when reductions were observed. The increase in tillering applied only to true tillers, however, and not to any other form of secondary growth, such as that from rhizomes. The effect of CCC on tiller production thus depended to a great extent on the inherent growth habit of the plant; a bunch type grass showed increased secondary growth, but a rhizomatous type showed a decrease. There also was less effect of CCC on tillering in the summer than in the winter. Possible explanations for the increase in tillering after CCC treatment and the apparent loss of the effect in the summer will be discussed later.

Root:shoot ratios indicated a trend, not observed in all experiments, for increase after treatment of plants with CCC. A possible reason for the somewhat variable and inconsistent results obtained, apart from difficulties in obtaining accurate dry weight data for roots, was that pots used for the greenhouse experiments became root bound towards the end of the experiment and this condition perhaps limited the expression of any effect that might have been caused by CCC. In view of Lockhart's findings (32) that CCC exerted little effect on the roots, it seems possible that the observed tendency for increased root:shoot ratio might have been due to decreased top growth concurrent with no change in root growth. The data obtained from the measurement of root length of wheat seedlings indicated that lack of effect of CCC on root growth could be the explanation for its effect on root:shoot ratio. The special experiment using 'bottomless' pots indicated, however, that measurements of root length versus top length make poor criteria for the study of root:shoot ratios, as root:shoot ratios obtained using root and stem length measurements did not correspond with those derived from dry weight data. This casts some doubt on the validity of the results obtained using measurements obtained from wheat seedlings. Before definite conclusions can be drawn regarding the effect of CCC on root: shoot ratios further experiments that can accurately determine root dry weight must be conducted; the use of hydroponic techniques with coarse sand, perlite or vermiculite as a rooting medium might be advisable.

The general conclusion from the data obtained for the total dry matter yield was that the lower doses of CCC, about

10 lbs./a. or less, had no effect or produced slight increases in dry weight, the magnitude of the latter depending on the time of year in which the plants were grown. Larger doses of 20 and 40 lbs./a. of CCC caused a reduction in yield. The increase in yield at the low rates of CCC was probably the result of increased tiller and leaf production, the reduction in height not being sufficiently large to inflict any loss of dry weight, but at the higher rates height reduction was so large that the increase in tiller and leaf number, if it occur-ed, could not compensate for the loss in weight due to stunt-ing. This was especially true for the species which appeared to be most susceptible to CCC, such as brome grass, barley and wheat. The observation that wheat seedlings grown in the dark in CCC solutions possessed greater dry weights than did similar plants grown in water suggested, as previously mention-ed, that CCC possibly had reduced the respiration rate slightly. No explanation can at present satisfactorily account for this, but if it is a general effect of CCC treatment, it would help explain why CCC-treated plants tended to have an increased dry matter yield. It could also supply one possible reason for the increased effects of CCC under winter conditions. The general assimilation rate of plants is low at that time of the year, permitting small differences due to lowered respirat-ion rate to become apparent. Much further work, however, would be necessary before any definite conclusions could be drawn concerning the influence of CCC on plant respiration rate and related changes in dry matter yield. An effect of CCC also related to dry matter yield became apparent with the discovery that plants grown in high concentrations of CCC

tended to have a higher percentage of dry matter; the implications of this discovery will be discussed later.

The results presented confirm the previous reports (53,21) that CCC seems to have less effect on plants grown during the summer than during the winter. The observed lack of interaction between CCC and light intensity recorded for the experiments conducted using a growth chamber suggested that light duration, or length of photoperiod, may be more important than light intensity. The results obtained from two of the experiments studying chlorophyll production in corn seedlings tend to support this suggestion. Increasing light intensity increased the effect of CCC on chlorophyll production. If this effect of CCC can be assumed to be indicative of its general interaction with light intensity, then it seems unlikely that the reduced effects of CCC in the summer in comparison with those produced in the winter could be explained on the sole basis of different light intensities. CCC-treated plants were also found to increase their chlorophyll content in the dark for about four days after exposure to light, in contrast with that of the controls which remained constant. This indicates the possibility that CCC-treated plants were able to respond to a relatively short photoperiod in the same manner that untreated plants would respond to a longer one, as chlorophyll production increases with the time of exposure of plants to light. Considerable further experimentation would be necessary before any definite conclusions could be drawn, but in view of the foregoing results and those of others (19,49,63,64) it seems possible that day-length, or photoperiod, may be more critical than light intensity in determining the magnitude of

the effects of CCC. Experiments involving different day-lengths in combination with CCC treatment, possibly also including accurately controlled different light intensities, would provide data of considerable value pertaining to the aspects of the effects of CCC discussed above. Since the winter experiments described in this thesis had supplementary lighting to maintain a day-length of 18 hours it could be argued that differences observed in the effect of CCC can be attributed to inherent random experimental error and not to the difference between summer and winter light conditions. There seems to be no doubt, however, that the observed differences were real, because the intensity of light from the relatively widely spaced 200 watt incandescent bulbs used to supply light during the winter could not have given an effect on vegetative growth equivalent to that of the sun during long summer hours.

Treatment of brome grass plants with CCC resulted in an increased chlorophyll content of the leaves in comparison with that of leaves from control plants. Subsequent work using corn seedlings grown in CCC solutions demonstrated that chlorophyll production was increased over that of plants grown in water. By incorporating ammonium ions, as ammonium chloride, into the culture solution the possibility that CCC itself was supplying additional nitrogen to the plants and thus enabling them to produce more chlorophyll was checked and eliminated. As previously mentioned, this also supplies further evidence that CCC is not broken down in the plant. Studies of protochlorophyll, the pigment generally considered to be the precursor for chlorophyll a (15,23,62), showed increased product-

ion, following CCC treatment, of the same magnitude as that recorded for chlorophyll. As suggested previously, this indicated that CCC could have increased the rate of metabolism at some point in the pathway leading to protochlorophyll synthesis. Further evidence that increased synthesis of chlorophyll and not a retardation of its destruction was the reason for the observed increases in chlorophyll content for plants grown in CCC solutions, was supplied by the experiment to investigate the rate of loss of chlorophyll in the dark. CCC had no effect or possibly slightly increased the rate at which chlorophyll was destroyed in the dark; therefore changes in the rate of chlorophyll destruction could not be expected to account for the observed increases in chlorophyll content recorded for the other experiments. The above experiment also indicated that synthesis of chlorophyll was increased, as CCC-treated plants continued to produce chlorophyll after being returned to darkness; the control plants did not possess this ability, which is in agreement with the findings for wheat reported by Virgin (57). An increase in chlorophyll content during growth in the dark is difficult to explain, as a previous report (56) indicated that chlorophyll production ceases after six to eight hours in the dark and that the level of protochlorophyll remains very low. The results obtained for protochlorophyll level presented in this treatise are in agreement with this. It is speculated therefore, that, as the conversion of protochlorophyll to chlorophyll _a_ requires exposure of the plants to light (23,24), CCC might enable the protochlorophyll, which has been demonstrated to be the photoreceptor for its own conversion reaction (23), to store, or

otherwise retain, the activated enzyme, or enzymes, and thus permit its conversion to chlorophyll a for a relatively long period in the dark. Although CCC might prolong the conversion of protochlorophyll to chlorophyll a after exposure to light, the majority of the increase in chlorophyll production that was found to occur for plants grown in CCC solutions would still appear to be the result of increased protochlorophyll synthesis, as this directly controls chlorophyll content during the early stages of the greening process (57). It is difficult to visualise how CCC could affect the pathway of chlorophyll synthesis (10,14,15) unless it can catalyse, or otherwise speed-up, one of the reactions involved. The possibility also exists that CCC does not affect the synthesis of chlorophyll, except in an indirect manner. Chlorophyll has long been known to exist in association with a lipo-protein complex in the plant (25). The accumulation of chlorophyll in the plant therefore depends on the production of this lipoprotein complex in addition to the synthesis of chlorophyll itself. Therefore, if the rate of production of the lipoprotein complex was a limiting factor, and it was increased by treatment of the plants with CCC, total chlorophyll content per plant would also be increased. Data obtained by Humphries (19) for the effects of CCC on tobacco indicated that there was a shift of nitrogen from the stems to the leaves, and he suggested that this might explain the changes in chlorophyll content. In view of the fact, however, that additional nitrogen supplied to corn seedlings did not effect such a large increase in chlorophyll content as that brought about by CCC, the indication is that changes in the nitrogen ratio

may not be important in determining the final chlorophyll content. The data available at present therefore do not indicate that the rate of production of the lipo-protein complex is important with regard to the effect of CCC on chlorophyll production. The possibility also exists that CCC affects the production of a compound common to the metabolic pathway involved in the production of tryptophan and of chlorophyll. Both chlorophyll and lipo-protein synthesis will be considered again when discussing the effect of CCC on 'free' tryptophan content. It can be concluded that the treatment of plants with CCC leads to their possessing a greater quantity of chlorophyll in comparison with that of untreated plants, and that this is associated with increased production of protochlorophyll, by a method which is not yet understood.

Free tryptophan was lower in wheat seedlings that had been grown in a CCC solution than in seedlings grown in water. It is of interest to note that these studies of tryptophan content, using a recording spectrophotometer, demonstrated that the technique developed by Fletcher and Zalik (9) can be applied to naturally occuring plant indoles other than IAA, provided that good chromatographic separation can be attained and accurate identification of the compound is carried out. As tryptophan has more than one function in the plant, the effects of changes in the amount present could be considerable. Tryptophan is now generally considered to be the precursor for IAA production (11,12,13), although the exact pathway, or pathways, of auxin biosynthesis still remain uncertain (13). CCC has already been shown to decrease the diffusible IAA level in pea stems (26) and a similar reduction has now been demonstrat-

ed for wheat seedlings. As IAA is produced from tryptophan, the reduction observed in the level of these two compounds is probably related. Halevy (16) demonstrated that CCC increased the rate at which IAA was broken down by IAA-oxidase in cucumber seedlings. If a similar effect occurs in wheat, then, as the level of IAA in the seedlings decreases, alteration of the equilibrium in the metabolic pathway for the synthesis of IAA from tryptophan in the direction of increased IAA production could occur, resulting in a decrease in the level of free tryptophan. This could only occur, however, if synthesis of tryptophan itself was not simultaneously increased. Arguments similar to those used above could also be used to show that such an increase would occur and would thus tend to invalidate the above argument. The difference in IAA content between equivalent amounts of CCC-treated and untreated seedlings was of the order of 0.1 to 0.2 µg., in comparison with a reduction of between 20 to 40 µg. for tryptophan. Halevy (16) recorded increases in IAA-oxidase activity of 3-to 6-fold, but to account for the results reported here the increase in activity would have to be approximately 200-fold. If increased IAA-oxidase activity was the reason for the observed decreases in tryptophan and IAA, then it would seem logical that relatively large quantities of an IAA break-down product should be produced; however, no large quantities of any other indole other than tryptophan could be detected on the chromatograms. On balance, the evidence makes it seem extremely unlikely that a reduction in IAA brought about by increased IAA-oxidase activity could account for the observed decrease in tryptophan. The possibility that the decreased

level of free tryptophan could account for the lowered IAA content should therefore be considered. If the tryptophan level was reduced by CCC treatment, then a similar reduction in IAA could be expected to occur. Increased IAA-oxidase activity could account for the observed larger effect of CCC on IAA level in the plant than on that of tryptophan. The observed reduction in tryptophan level could be brought about in one of three ways: 1), by partial blocking of its synthesis, 2), by increased incorporation into proteins and 3), by increased rate of destruction. These possibilities have not been investigated. If CCC exerts its effects on early tryptophan precursors it is conceivable that in the resulting complex changes in the metabolic reactions a partially blocked reaction could produce an accumulation of a compound necessary for chlorophyll production, either for the synthesis of chlorophyll itself or to supply a limiting factor in the production of the chloroplast lipo-protein complex. Thus the two apparently unrelated effects of CCC on chlorophyll and tryptophan production could be more easily explained. At this stage it is clear only that the discovery that CCC affects the level of free tryptophan in the plant can induce considerable speculation concerning the site of action of the chemical.

Reduction in the level of IAA in CCC-treated wheat and pea (26) plants is of significance in relation to explanations of some of the morphological effects observed after treating plants with CCC. Although there is considerable doubt concerning the role of auxin in controlling apical dominance, as reviewed by Audus (1), the old theory that the flow of IAA through the tissues governs the development of lateral buds

has yet to be disproved. Therefore, a reduction in IAA level resulting from treatment of the plants with CCC could lead to increased lateral bud development; this would provide an explanation for the increases in tillering observed for several species investigated, and also for the lack of effect recorded for brome, except in the early stages of development, which relies mainly on shoots produced from rhizomes rather than from lateral buds for secondary vegetative development. The effect of CCC on stem length is more difficult to explain in terms of IAA level. If the primary action of IAA is considered to be controlling cell elongation, then decreased cell elongation would be expected to result from treatment of plants with CCC. Previous workers have shown that the reduction in internode elongation after treatment of plants with CCC was due to decreased subapical cell division and not to reduced cell elongation (43,44,63). However, if the concept of auxin action proposed by Kefford and Goldacre (20) does explain the mechanism governing cell division and elongation, then the observed effects of CCC on stem elongation can be explained through changes in IAA level. These authors reason that the action of IAA on cells is through a predisposing agent, which, in the presence of gibberellin causes elongation, and in the presence of kinin causes division, and that under conditions of limiting amounts of auxin there could be competition between the two processes, the outcome depending on the balance of gibberellin and kinin. Thus, if this balance is such that in normal plants a reduction in IAA affects the predisposition of kinin to a greater extent than that of gibberellin, then decreased cell division would occur (as has been described for CCC-treated plants), resulting in shorter stems.

This would explain reported data showing that relatively small doses of IAA reversed the effects of CCC, while considerably larger relative doses of gibberellin were required to produce a reversal of a similar magnitude (26,63). The above hypothesis does not appear to be the final answer, however, as it is difficult to see why only sub-apical and not apical cell division was affected; this apparent anomaly cannot be resolved by the data available at present. The above hypothesis, however, also appears relevant in explanation of the apparent antagonism of the effects of CCC and those of gibberellin. The visible effects of CCC application on plants have been shown to be opposite to those of gibberellin (e.g., 32,53,54,60), but no definite conclusion has been drawn concerning the reasons for this. The results discussed above tend to confirm the opinion of Kuraishi and Muir (26) and more recently Cathey (4) that CCC is not an antagonist of gibberellin in the metabolic sense. As far as can be ascertained there has been no report of gibberellin affecting the level of free tryptophan in plants. It is therefore difficult to visualise how the effects of CCC on both tryptophan and IAA level in the plant could be due to changes originally induced in gibberellin. Changes in the level of IAA, however, in combination with gibberellin and kinin, as suggested previously, might be capable of producing the observed apparent antagonism. The effects of CCC on flower initiation and flower production, as reported by several authors (48,58,60,63,64) may possibly be connected with the observed changes in IAA level, which, according to a review by Lang (28), is closely associated with the flowering process. Results of the present studies were not applicable

to any further correlations regarding this aspect of plant growth.

CCC was found to have relatively little effect on the morphological development of oat plants. Spectrophotometric determinations of extracts from oat seedlings indicated, although extensive testing was not carried out, that the level of an indole compound (probably IAA or tryptophan) was not affected by treatment of the plants with CCC. This suggests, in the light of the results described in this thesis, that the response of plants to CCC may be closely related to the level, and the rates of synthesis, of their native indoles.

Some of the data presented in this treatise may help in explanation of effects of CCC that other workers have reported. Van Emden (55) found that the aphid population of CCC-treated brussel sprouts was lower than that of untreated plants, and possibly related to this was the increased tolerance of CCC-treated tomato plants to Verticillium wilt reported by Sinha and Wood (46). In both cases the authors suggested that changes in the host plant physiology, rather than that of the parasite, could be the reason for the observed effects. Reduction in the level of free tryptophan in the host plants could explain these effects, especially that observed for aphids on brussel sprouts. Tryptophan is an essential amino acid for members of the animal kingdom. This means that it must be supplied in their diet, as they are unable to synthesise it for themselves. A smaller quantity of this amino acid in the plant would mean that the aphids would not be able to grow and multiply as successfully as those on plants not treated with CCC. This explanation agrees with the findings

reported by Van Emden. The observed reduction in water content in corn seedlings grown in CCC offers a possible explanation for the report by Miyamoto (37) that CCC increased the tolerance of wheat seedlings to high salt concentrations in the growing medium. Reduced water content could be manifested by increased concentration of the cell sap, although no evidence was obtained to suggest that this was indeed the case. If the cell sap were more concentrated in CCC -treated plants than in those which were not, then the increased osmotic presure would enable treated plants to survive and grow under conditions of salt concentration normally too high for healthy growth. Although Miyamoto did not discuss practical applications of his work, it would seem to the present author that if treatment of plants with CCC increases their ability to withstand high salt concentrations, it might find practical application in the establishing of crop plants in areas of high salinity. It is also possible that increased cell sap concentration after treatment of plants with CCC, if it occurs, could explain the increase in drought resistance reported by Halevy and Kessler (17). A higher cell sap osmotic pressure would enable the plants to extract more water from the soil. This was reported by Halevy and Kessler; the soil in which CCC-treated bean plants had been grown contained less water at the wilting point than did soil growing untreated plants. Greater root development, however, might accomplish the same effect, and thus no conclusion can be drawn without further experimentation.

From the general practical standpoint CCC does not appear

to have any large scale commercial value concerning the induction of quantitative effects on grasses. On the other hand it appears to have far reaching qualitative effects on plant metabolism and physiology, deserving additional study of potential basic and practical significance.

SUMMARY

1. Results from application of CCC using foliar spray techniques were approximately similar to those previously reported following use of soil treatment.

2. CCC tended to increase the ability of grasses to recover from clipping, and also possibly their resistance to drought, but did not produce any substantial effects of practical value on grasses grown under field conditions in these experiments in Alberta.

3. Height was reduced after treatment of the plants with CCC. Closely related to this was a reduction in rhizome length produced by plants treated with CCC. In both cases the magnitude of the effect increased with increasing CCC dose.

4. Tiller production per plant was generally increased by treatment of plants with CCC. At high doses, however, the general retardation of growth by CCC was sufficiently great to also cause a reduction in tillering, especially on the more sensitive species such as wheat and barley.

5. Total leaf number per plant was increased by CCC treatment if tillering was increased, but the number of leaves per apex showed no significant differences.

6. The effect of CCC on height, leaf number and tiller production appeared to decrease with time, possibly because of a dilution of CCC within the plant.

7. Considerable differences in response to CCC occurred between different grass species; in general the short stemmed bunch type grasses were affected less than the long stemmed rhizomatous types.

8. Oats appeared to be less responsive to treatments with CCC than wheat and barley, while brome was the most sensitive of the forage species investigated.

9. Root:shoot ratios were generally increased by treatment of the plants with CCC. The possibility that this effect results from a combination of lack of effect on roots concurrent with decreased top growth is unresolved.

10. Total dry matter yields were generally slightly increased at the low levels of CCC and decreased by high levels, the magnitude of the effect depending on the species and the time of year during which the experiment was conducted.

11. The effect of CCC appeared to be greater in the winter than in the summer; light intensity and effect of CCC showed no significant interaction and the suggestion is made that length of day, or photoperiod, may be more critical in determining the magnitude of the effect of CCC than light intensity.

12. Dark-grown CCC-treated wheat seedlings appeared to have a higher dry matter content than untreated seedlings.

13. Corn seedlings grown in high concentrations of CCC had a lower water content than those grown in water only.

14. Plants treated with CCC generally appeared to be greener than untreated ones, and brome grass leaves from treated plants contained more chlorophyll than did leaves from untreated plants.

15. The production of chlorophyll by corn seedlings grown in CCC solutions was increased over that of seedlings grown in water. This effect appeared to be due to an increased rate of protochlorophyll synthesis.

16. Use of the technique for measuring IAA content developed by Fletcher and Zalik (9) was feasible for measurement of the free tryptophan content of wheat seedlings, provided accurate identification and adequate chromatographic separation was carried out.

17. The free tryptophan and IAA content of CCC-treated wheat seedlings was lower than that of untreated seedlings. The possible relationship of the biosynthetic pathway involved in the production of these two compounds is discussed, and potential consequences of reductions in their levels in relation to plant morphological development and to insect and disease resistance are considered.

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